1994

# Instrumental and Sensory Characterization of the Flavor of Blue Crab (Callinectes Sapidus) Meat and Processing By-Product.

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Order Number 9502105

# Instrumental and sensory characterization of the flavor of blue crab (*Callinectes sapidus*) meat and processing by-product

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The Louisiana State University and Agricultural and Mechanical Col., 1994

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# INSTRUMENTAL AND SENSORY CHARACTERIZATION OF THE FLAVOR OF BLUE CRAB (*Callinectes sapidus*) MEAT AND PROCESSING BY-PRODUCT

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by
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May 1994

**ACKNOWLEDGMENTS**

The author wishes to express his sincere and deepest appreciation to his present advisor, Dr. Keith R. Cadwallader for his inspirational and creative advice as well as his encouragement and patience throughout the years. Appreciation is also extended to his former advisor, Dr. Thomas C.-Y. Hsieh, for his tremendous technical support and guidance during his tenure in the Department of Food Science.

Appreciation is expressed to Dr. Auttis Mullins, Professor and Head, Department of Food Science, for his support of this research. Sincere thanks are given to Dr. Samuel Meyers, Dr. J. Samuel Godber, Dr. Robert M. Grodner, Dr. Paul W. Wilson, and Dr. Richard E. Condrey (Dean's Representative) for serving on the dissertation committee and for their valuable assistance and constructive advice.

Gratitude and appreciation is extended to his closest friend, Mr. Tobun Cheung, for his encouragement and support. The author wishes to thank all the sensory evaluation panelists for their excellent sensory skills during the course of training and experimentation. Special thanks are given to Mr. Feng Chen for his assistance in portions of the experiments.

Finally, the author expresses his deepest indebtedness to his parents and his brothers for their countless encouragement, patient, understanding, love and prayers. It is to them that this work is dedicated.

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| | |
|---|---|
| 2-AP | 2-acetyl-1-pyrroline |
| AEDA | aroma extract dilution analysis |
| amu | atomic mass unit |
| A-SDE | atmospheric simultaneous steam distillation-solvent extraction |
| CPB | blue crab processing by-product |
| BX | bone extract |
| BD | 2,3-butanedione |
| C | control (distillation) |
| C' | control (reflux) |
| (E) | *trans* geometric configuration of an organic molecule |
| E | experimental (distillation) |
| E' | experimental (reflux) |
| FTIR | fourier transform infrared spectrometry |
| FID | flame ionization detector |
| FD | flavor dilution |
| FSOT | fused silica open tubular |
| GC | gas chromatography |
| HPT | (Z)-4-heptenal |
| IRD | infrared detector |
| IS | internal standard |
| 3-MP | 3-(methylthio)-propanal |
| MCT | mercury cadmium telluride |
| m/z | mass/charge ratio |

| | |
|---|---|
| MSD | mass selective detector |
| MS | mass spectrometry |
| O | olfactometry |
| PID | photo-ionization detector |
| PYR | pyrrolidine |
| RSD | relative standard deviation(s) |
| RE | residue |
| RI | retention index |
| V-SDE | vacuum simultaneous steam distillation-solvent extraction |
| WX | rinse water extract |
| SIM | selected ion monitoring |
| SP | supernatant |
| TIC | total ion chromatogram |
| V | voltage |
| (Z) | *cis* geometric configuration of an organic molecule |

**ABSTRACT**

Blue crab (*Callinectes sapidus*) meat and its processing by-product (CPB) were characterized by instrumental, olfactometric, and sensory techniques to determine the usefulness of CPB as a feed stock for flavor recovery. Instrumental analysis with gas chromatography/mass spectrometry (GC/MS) and gas chromatography/Fourier transformed infrared spectroscopy (GC/FTIR) allowed for the identification of 77 and 80 compounds in claw meat and CPB, respectively. Fifty-five compounds were found common to both samples, suggesting that CBP could be a good source for flavor recovery. Quantitatively, trimethylamine (TMA), alkanes (C15-C17, C19), and indole were high (>50ng/g) in claw meat while TMA, carbon disulfide, dimethyltrisulfide, alkanes (C15 and C17), geranylacetone, and 1-dodecanol, among other detected compounds, were high in CPB. A subsequent experiment to define claw meat aroma was carried out by analyzing extracts prepared by atmospheric (A-SDE) and vacuum simultaneous steam distillation-solvent extraction (V-SDE) using gas chromatography/olfactometry (GC/O). After data reduction process, the randomly occurring odorants among the replicated samples were eliminated. Claw meat aroma was defined by seven common odorants, of which five were identified as: 2,3-butanedione,

pyrrolidine, (Z)-4-heptenal, 2-acetyl-1-pyrroline, and 3-(methylthio)-propanal. A later experiment involving sensory evaluation confirmed that the aroma of claw meat differed from that of lump meat. The results were in agreement with GC/O findings. Overall results indicated that three odorants were indispensable in crabmeat aroma and could serve as markers for evaluating CPB flavor quality. These markers included pyrrolidine, 2-acetyl-1-pyrrolidine, and 3-(methylthio)-propanal. By monitoring the occurrence and the amount of these markers, it was confirmed that CPB was a good source of flavor recovery. Optimal recovery of these markers could be achieved by heating. Distillation was found to be superior to reflux in recovering the markers.

# CHAPTER 1

## INTRODUCTION

Commercial blue crab processing comprises a significant portion of the Louisiana seafood industry (Jaworski, 1972). In 1986, 27 million pounds of blue crab were processed, of which about 11% by weight of picked meat was recovered (Keithly et al., 1988). The remainder was disposed of as processing by-product.

With rising concerns for a cleaner environment, food processing by-product cannot be released into the environment without pretreatment. Because of this, utilization of such by-product has become more popular in the past few years. This is exemplified by the recovery of astaxanthin pigment from crawfish processing by-product (Meyers, 1987) and successful incorporation of crab mince recovered from blue crab processing by-product (CPB) into a food-product formulation (Lee et al., 1993).

Total product utilization has been an important area of study, especially in view of present environmental issues such as the limited use of landfills for disposal of crab processing scrap as a result of concerns of possible groundwater contamination (Genetelli and Cirello, 1976). CPB utilization has the potential to benefit the processor financially and may offset the actual cost of

waste disposal. Since processing of crab is a year-round operation in Louisiana, the industry needs to acquire the proper technology for alternative use of CPB. One option is to recover the flavor components from CPB.

To utilize CPB as a food-grade flavoring material, the quality of the by-product has to be determined and compared with fresh crabmeat. Although there have been a number of articles on volatile components detected in crab (Rayner et al., 1981; Ando and Osawa, 1988; Hsieh et al., 1989; Flament, 1990; Matiella and Hsieh, 1990; Cha et al., 1993), there was little indication as to which volatile compounds were important contributors to cooked crabmeat aroma. In addition, some crab connoisseurs claim that the flavor of meats from various parts of the crab is different. The problem becomes more complicated because the literature has not established compounds that could be used as markers to screen the quality of CPB.

Analytical instruments in general are very useful in identifying unknown compounds, but they lack the ability to indicate desirability or descriptive quality. This deficiency has a profound effect in the analysis of samples with flavor quality as the major concern. Fortunately, this problem has been solved with the development of a technique often used in aroma research known as gas chromatography/olfactometry (GC/O) (Acree, 1993). This technique bridges the gap between analytical

instrumentation and the human olfactory sense. The human olfactory organ is very sensitive to odorous compounds and sometimes exceeds the detection limits of other common detectors (Cain, 1977). Results from GC/O enable researchers to pinpoint the time and the quality of an odorant as it elutes from the GC. This technique could be useful in defining authentic blue crab aroma as well as its processing by-product.

One essential, but always overlooked, consideration for recovering flavor was the freshness of the raw material (Meyers and No, 1994). In order to avoid any undesirable changes in the CPB which may end up in the final flavorant, the raw material should be utilized as early as possible in the crab processing operation.

The correlated objectives of this study were: (1) to compare the volatile components in blue crab claw meat and its processing by-product (Chapter 3); (2) to define blue crab claw meat aroma by GC/O (Chapter 4); (3) to evaluate the differences between two types of crabmeats (claw vs lump) by sensory evaluation and GC/O (Chapter 5); and (4) to evaluate blue crab processing by-product as a potential source for flavor recovery (Chapter 6).

# CHAPTER 2

# REVIEW OF LITERATURE

## Crab

Crabs can be classified into two groups as true (*Brachyura*) and hermit (*Anomura*) crabs (Warner, 1977). The small, symmetrical, and flaplike abdomen is found bent forward in the ventral surface of the thorax in true crabs, but is long, asymmetrical, and often twisted in hermit crabs (Bowman, 1986). As with other crustaceans, the crab's body is divided into three parts, namely head, thorax, and abdomen. The head and the thorax are fused together to form the cephalothorax. And its body is protected by a hard carapace of exoskeleton (Schmitt, 1965).

There are 4500 species of true crabs found throughout the world. Most dwell in the sea, but some inhabit fresh water and terrestrial areas (Warner, 1977). They range in size from as small as the pea crab (family *Pinnotheridae*) of less than 1.5 mm to the large Japanese spider crab (*Macrocheira kaempteri*) measuring 3.5 m from leg tip to leg tip (Warner, 1977).

For the most part, crabs are scavengers and eat a variety of food, yet some are vegetarians (Warner, 1977). Crabs can survive on small fish, worms, and plankton. Some

terrestrial crabs feed on special diets. For example, the tree-climbing rubber crab feeds on coconut meat and the tree crab feeds on mangrove leaves (Warner, 1977). To understand how crabs can adapt so well to the environment, their specialized anatomy has to be examined.

## Anatomy

Nineteen pairs of appendages, in the order of five, eight and six pairs, can be found on the head, the thorax and the abdomen, respectively, of true crab (Schmitt, 1965). Of those on the head, the first two pairs form the first and second antenna as scenting devices (Bowman, 1986). The rest are mouthparts, including mandibles and maxillae for food handling, as well as for gill cleaning and venting (Bowman, 1986).

Maxillipes (three pairs on the thorax)) also serve as food-handling and respiration devices. Five pairs of pereopods form the walking legs, usually with the first pair modified to large claws known as pincers or chelipeds (Bowman, 1986). True crabs rarely contain all six pairs of abdominal appendages including pleopods (swimmerets) and rudimentary uropods (tail fan). More often, only pleopods are present--two pairs in male as copulatory organs and four in female as attachments for eggs. One distinct feature of the true crab is the fused part formed between

the epistome and the head sides of the carapace (Bowman, 1986).

## Taxonomy and Life Cycle

Crabs belong to the phylum Arthropoda, class Crustacea, subclass Malacostraca, order Decapoda, suborder Pleocyemata, and infraorders Brachyura and Anomura (Warner, 1977).

The development of true crab involves four stages (Bowman, 1986). The first two stages, nauplius and protozoea, occur in the egg, followed by hatching during the zoea stage. Zoea larva pass through the megalops stage before being transformed into adult crabs. A number of moltings and sheddings of the exoskeleton occur during the growth period. A typical life cycle, e.g., in blue crab, *Callinectes sapidus*, involves eggs hatched to free swimming zoea larva; molting of zoea larva and their transformation into megalops larva containing tail-like abdomen; and development and molting of megalops larva to immature adult crabs that continue to grow and molt until reaching mature size (Jaworski, 1972).

Among the various crab species, several are considered important commercial resources, especially in the United States. These include the king crab (*Paralithodes camtschatica*), the blue crab (*C. sapidus*), the Dungeness crab (*Cancer magister*), and the snow crab

(*Chionoecetes bairdi* and *C. opilio*) (O'Bannon 1993a and b). In Louisiana, blue crab is the major species caught in the Gulf Coast region.

## Crab Industry in Louisiana

In 1992, there were 624.3 million pounds, valued at $471.3 million, landed of all species of crabs in the United States. Landings of hard blue crab amounted to about 31% of the total landings (O'Bannon, 1993a and b). Harvesting of blue crab is among the most important commercial fishery operations in the southeastern United States. Crabbing has been an important industry in Louisiana since the middle 1920's when the first crab processing plant began operation in Morgan City (Jaworski, 1972). Most crab processors either boil (for 15-20 min) or retort (at 121°C for 9-20 min) live crabs, after which the carapace of each crab is manually removed (Moody, 1974; Babbitt, 1992). The debacked crabs are cleaned before overnight storage in crates. Manual removal of meat from the debacked crabs is carried out the following morning. Three types of crabmeat are picked: lump, white meat, and claw meat. Lump meat, which is the most valuable of the three, is picked from the body cavity near the swimming legs. The white meat from the walking- leg chamber and claw meat are collected separately (Ingram and Moody, 1990). Other than the edible parts produced during the

picking operation, a substantial amount of by-product is also produced, which often includes the hard tissue, guts, gills and a large portion of meat.

## Crab Processing By-Product

Every year, several hundred million pounds of by-product are generated nationally by the blue crab processing industry accounting, for almost 90% by weight of the total weight of processed blue crabs (Keithly et al., 1988). Moreover, liquid wastes produced from cooking or rinsing processes contain substantial amounts of protein (Hanover et al., 1973), which drastically increases the biological oxygen demand (BOD), chemical oxygen demand (COD), and total organic carbon (TOC) values compared with the original wash water (Hanover et al., 1975). Thus, substantial liquid waste can be produced.

On the other hand, proper disposal of such food processing wastes has been a major problem for the food industry. With stricter regulations and environmental protection enforcement, food processors have had to find ways to counter-balance the high disposal cost of their processing wastes. Proper by-product utilization has become a very important area of investigation.

Significant technology has been developed for the utilization of crayfish processing by-product for recovery of carotenoid astaxanthin (Meyers, 1987). Recently, Cha et

al. (1992) prepared flavor concentrates from crayfish processing by-products by evaporation at 85° and 100°C and reported that more heterocyclic flavor compounds were produced at 100°C. Lee et al. (1993) were able to incorporate crab mince recovered from undersized crab claw into valuable crab-related food products. Nieto et al. (1989) explored the production of a flavor concentrate from mechanically recovered CPB. However, no experimental information was given by these investigators. Jaswal (1990) prepared a high-quality amino acid hydrolysate from CPB by varying hydrolysis time and acid concentration. Kim et al. (1994) added hepatopancreas enzyme prepared from crayfish to CPB and suggested that the enzymatic hydrolysis increases the concentration of flavor precursors in CPB, which could lead to its potential use for flavor recovery. Recently, Cha et al. (1993) reported the feasibility of preparing flavor extracts from the effluent from cooking snow crab. Another alternative way to utilize CPB is to extract the volatile flavor components or aroma precursors to produce a food-grade flavoring if CPB can be proved to be a good feedstock for such purpose. Nevertheless, a review of the literature did not show any previous works done to evaluate the usefulness of CPB as a source for flavor recovery.

## Composition of Crab

Before understanding what produces the desirable crab aroma, it seems that a general understanding of the proximate composition of crabmeat is necessary. George and Gopakumar (1987) reported marked differences in the biochemical composition of crabmeat from the body and claw of female crab (*Scylla serrata*). Both meats had relatively high amounts of glycogen and phosphorous. Claw meat contained a slightly higher amount of ribose sugar and a much higher amount of sodium ion than the body meat, which contained more minerals such as potassium and calcium. Sarcoplasmic protein content was higher in body meat, while myofibrillar protein content was higher in claw meat. Glycine and alanine were the major components in the amino acid pool. Other major amino acids found in the body meat included lysine, aspartic acid, and histidine, while the claw meat contained valine, histidine, leucine, and phenylalanine.

Siddiquie et al. (1987) studied the composition of three species of crab, *Portunus pelagicus*, *P. sanguinolentus* and *Scylla serrata*, found in Pakistan. All three species contained dry tissue ranging from 85-95% organic matter with 55-65% protein. The percent dry weight of lipids, carbohydrates, and ash were small. Table E.1 shows the average percent wet weight composition of several species of crab. As noted by the authors, the age,

size, and sex of the individuals, as well as the seasonal and environmental variations, contribute to variability among species. In a one-year study of the proximate composition of Chesapeake Bay blue crab, Farragut (1964) observed fluctuations in the content of moisture, protein, fat, and ash in the body, claw, and offal tissues, which coincided with the mating and spawning seasons. These variations may have an indirect impact on the cooked crab aroma and overall acceptability of crabmeat, since the amount of precursors involved in aroma production may vary. Table E.2 shows the proximate composition of cooked crabmeat. Since liquid is lost during cooking, the moisture content is lower in cooked crabmeat (Table E.2) than in raw crabmeat (Table E.1). Increases in the percentage of protein, fat, and ash in cooked meat may also be the result of the decreased moisture content.

**Formation of Volatile Flavor Components in Crab**

Recently, Hayashi et al. (1990) suggested that the aroma of cooked crabmeat was generated from non-volatile extractive components in the raw sample. They reported that the non-volatile components, which decreased during heating (especially in the temperature range of 85° to 100°C), might be involved in the generation of crab flavor. They further suggested that certain amino acids, in high concentration in the original extract, might be

precursors of the cooked-crab flavor. So far, there are no reports of one single character-impact compound being responsible for crab flavor. A similar situation is found in the identification of cooked-beef aroma. Most of the volatile compounds in beef are generated via Maillard reactions (Liu et al, 1987). Characteristic aromas that distinguish various cooked meats from different species, such as beef, pork, mutton, etc., are believed to involve fat, even though its role may not be the same in all meats (Wasserman and Tally, 1968). Wasserman and Tally (1968) investigated the influence of fat on the organoleptic identification of several roasted meats and concluded that beef fat was not critical in beef aroma, while pork and lamb fats might contain factor(s) that generate the characteristic aroma of each meat during roasting.

Contrary to the idea that fat is important in cooked meat, Pippen et al. (1969) suggested that fat, particularly with poultry, acted only as a medium in retaining the dissolved aroma from the cooked lean portion of meat. This may also be true with seafoods. In investigating the volatile compounds of cooked shrimp, Kubota et al. (1989) identified two unsaturated methylketones, (Z,Z,Z)- and (E,Z,Z)-5,8,11-tetradeca-trien-2-one, that retained a weak, but characteristic seafood aroma similar to that found in shrimp, crab, shellfish, sea cucumber, etc. The mechanism(s) of the

generation of the two ketones was not known, but the investigators observed that these volatile compounds were only produced from the defatted residue of small shrimp in water and not from the lipid portion. Furthermore, the amount produced increased at higher temperatures (e.g. >80°C) and longer times. Since the onset of protein denaturation was noted at 80°C, Kubota et al. (1989) suggested that the formation of methylketones was related to the protein fraction. Recently, Boyle et al. (1992) proposed that the presence of a small quantity of bromophenols was critical in the desirable brine-like or sea-like flavor note in most saltwater seafood, although others have found them to be the major cause of off-flavor in Australian prawns (Whitfield et al., 1988). Boyle et al. (1992) detected these compounds consistently in most saltwater species but not in freshwater fishes. They believed that these compounds might originate in the food source.

Overall, crab flavor may consist of products that are (1) produced from heating; (2) acquired from the environment; and/or (3) synthesized metabolically by the organism. Present knowledge of the identified volatile compounds supports the hypothesis that important and characteristic crab flavors are thermally generated, although environmentally acquired compounds, such as bromophenols from feed, may also be important to overall

crab flavor. However, the significance of these consumed compounds to the overall crab flavor can only be confirmed if their threshold values are small while their concentrations are relatively large. Finally, the possibility that metabolites significantly contribute to a characteristic crab aroma seems unlikely, judging from the qualitative data presented in Table E.3.

## Crab Flavor

Flavor is the sensation perceived by both sensory structures of the olfactory glands and the taste buds. The former deals with sensation produced by volatile compounds, while the latter involves primarily nonvolatile compounds. Volatile components can be recovered and analyzed by means of distillation and extraction, followed by identification, using gas chromatography and gas chromatography/mass spectrometry (Shye et al., 1987). The major groups of volatile components from seafood such as shrimp include pyrazines, ketones, pyridines, alcohols, furans, and hydrocarbons (Shye et al., 1987). By using a dynamic headspace sampling (DHS) system, Matiella and Hsieh (1990) detected 53 compounds in both boiled and pasteurized blue crab, including aldehydes, ketones, alcohols, aromatics, furans, sulfur-containing compounds, terpenes, alkanes, and other miscellaneous compounds.

Elsewhere, Japanese investigators have reported the presence of 50 compounds, including esters, aldehydes, alcohols, sulfur-containing compounds, pyrazines, pyridines, oxazoles, furans, pyrroles, and other miscellaneous compounds in the first and second distillation fractions from blue crab shell and meat (Ando and Osawa, 1988). Similarly, Cha et al. (1993) identified 122 volatile compounds in both snow crab cooker effluent and its concentrate.

Table E.3 summarizes the volatile flavor compounds identified in blue crab, *Callinectes sapidus*. Nineteen alcoholic constituents were identified by Flament (1990) in the neutral fraction from a powdered crab-flavor extract. Alcoholic components from Flament (1990) were included, although they were identified in a crab flavor extract of an unspecified species. The compounds have been divided into 10 classes, namely, aldehydes, ketones, alcohols, aromatics, furans, sulfur-containing compounds, terpenes, alkanes, pyrazines, and miscellaneous compounds. The largest group in this table is the alcohols, while the smallest group is the terpenes. Groups such as aldehydes, ketones, alcohols, and alkanes could have originated from lipids via oxidation and degradation (Tressl, 1990). Groups involving heterocyclic rings, such as furans and pyrazines, might be formed via the Maillard reaction (Tressl, 1990). Even though abundant qualitative data are

available in the literature, little information has been given to indicate which of these compounds are major contributors to crab aroma, i.e., compounds that can characterize the basic crab flavor have yet to be determined.

## Meaty Flavor from Model Systems

Numerous papers and patents have covered the subject of cooked-meat aroma. It has been established that heating low-molecular-weight precursors from meat leads to an increase in cooked aroma impression (Hornstein and Crowe, 1960). Moreover, it is recognized that the presence of sulfur-containing compounds is essential to meaty aroma. A simple model system designed to study roasted-meat flavor was carried out by van den Ouweland and Peer (1975). The reaction involved reacting 4-hydroxy-5-methyl-3(2H)-furanone and its thio analog with hydrogen sulfide. Both furanone and the analog are sugar degradation products and are found in beef broth. Hydrogen sulfide was produced from cysteine in muscle during roasting. Groups of volatiles having beef aroma were detected, including furans, thiophenes, alkanones, furanones, and dihydrothiophenes.

Similarly, the substitution of sulfur at the 3-position of furans produced cooked or roasted-meat aroma compounds such as 2-methyl-3-furanthiol, 2,5-dimethyl-3-

furanthiol. All have low threshold values (Evers, et al. 1976).

Shibamoto and Russel (1976) studied a D-glucose-hydrogen sulfide-ammonia system in relation to meaty flavor. Twenty-four compounds were identified, including various sulfides, thiophenes, ketones, thiazoles, furfurals, and furans. Sugars, hydrogen sulfide, and ammonia were suggested to be important reactants. Both ammonia and hydrogen sulfide were formed from Strecker degradation reactions. In addition, hydrogen sulfide could be formed by heating meat in the presence of thiamine.

Hwang et al. (1986) identified the volatile compounds generated from the reactions of selected aldehydes with ammonium sulfide in order to understand the flavors generated from fried chicken and french-fried potatoes. They suggested that the flavors were formed via nonenzymatic browning reactions, degradation of sugar, thermal and oxidative decomposition of lipids, lipid-protein interactions, and degradation product interactions. The groups of compounds identified included pyridines, trithiolanes, amines, alkenals, alkenoic acids, sulfides, disulfides, alkenals, dithiazines, and trisulfides.

The presence of sulfur-containing amino acids, i.e., cysteine, cystine, and methionine, are essential precursors in the production of meaty aroma (Tressl,

1990). However, the presence of suitable simple carbohydrates under the proper conditions also is critical for production of the flavor.

Recently, the thermal degradation of thiamin was described as important for the formation of cooked and roasted meat aroma. The thiamin degradation pathway has been reviewed by Güntert et al. (1992).

## Aroma Extract Dilution Analysis (AEDA)

AEDA is one of several techniques used in the gas chromatography/olfactometry (GC/O) method to evaluate odor activities of volatile compounds in a sample extract. Based on the type of treatment of the data, the GC/O method is divided into three major groups, namely, dilution analysis, cross-modal matching, and perceived intensity (Acree, 1993).

Dilution analysis includes two techniques--AEDA (Ullrich and Grosch, 1987) and CharmAnalysis™ (Acree et al., 1984). Both are similar in principle, but differ in the manner in which the data are collected and processed. The latter technique represents an advanced technology compared with the former. CharmAnalysis™ requires a software program, Charmware™, in addition to the hardware system. Serially diluted samples, usually in a 1:1 or 1:2 dilution ratio, are prepared for both techniques. In

either case, the success of dilution analysis depends heavily on how the initial flavor extract was prepared.

AEDA has been used widely as a screening technique for important flavor compounds involved in: (1) the development of off-flavor in food, such as soybean oil, boiled beef, butter oil, and linoleic acid (Ullrich and Grosch, 1987; Konopka and Grosch, 1991; Widder et al., 1991; Guth and Grosch, 1992); (2) foods that underwent thermal process as in Arabic coffee, cooked beef, and roasted beef (Gasser and Grosch, 1988; Blank et al., 1992; Cerny and Grosch, 1992); and (3) spices, including parsley leaves and olive oils (Guth and Grosch, 1991; Jung et al., 1992).

The results of AEDA are expressed as a flavor dilution (FD)-factor, which is defined as "the ratio of the concentration of a compound in the initial extract to its concentration in the most dilute extract in which the odor was still detected by GC/O" (Blank et al., 1992). In fact, the FD-factor is the dilution factor of the initial extract.

Recently, Abbott et al. (1993) compared the techniques used in AEDA and CharmAnalysis™ reporting that both have similar intrinsic problems, such as variation of results between subjects, and within subjects and the appearance of gaps in responses, etc. These researchers pointed out that CharmAnalysis™ could better determine

the contribution of an odorant, but they did not indicate which technique was superior. The recommendation was to obtain data from both methods and to evaluate each.

# CHAPTER 3

## VOLATILE COMPONENTS IN BLUE CRAB (*Callinectes sapidus*) CLAW MEAT AND PROCESSING BY-PRODUCT

### Introduction

Commercial blue crab processing is an important seafood industry. By-product that remains after processing comprises as much as 85% of total crab weight and may be processed into crab meal or, more often, discarded in landfills. Environmental awareness and regulations have encouraged alternatives for by-product utilization. Value-added products, such as mechanically extracted minced meat, have been successfully recovered from crab picking-table by-product (Gates and Parker, 1992; Lee et al., 1993). Jaswal (1990) demonstrated that amino acid hydrolysate could be produced from crab processing by-product. Utilization of crab processing by-product for volatile flavor recovery and flavor extract production should be investigated. Studies on crab flavor have focused primarily on the non-volatile components (Konosu et al., 1978; Hayashi et al., 1979; 1981), with fewer studies concerned with the volatile portion (Hsieh et al., 1989; Flament, 1990; Hayashi et al., 1990; Matiella and Hsieh, 1990). However, little information has been published about the flavor quality of the by-product

compared with crabmeat itself. The objective of this research was to compare the volatile-flavor profiles of fresh-picked blue crab claw meat and picking-table blue crab by-product (CBP) to assess the potential use of the CBP for flavor recovery.

## Materials and Methods

### Materials

Vacuum-packaged, fresh-picked crab claw meat and CBP (picked crab cores with attached legs, carapace, gills, etc.) were obtained from a local processor in Baton Rouge, Louisiana. Three separate sample collections were made in August, 1991. Materials were kept on ice and immediately transported to the LSU Muscle Foods Laboratory within 1 hr of processing. The CPB was then ground using a meat/bone separator (Paoli, Model 23 668, Stephen Paoli International, Rochford, IL); homogenized using a silent mixer (Model 84181D, Hobart, Troy, OH); and then vacuum-packaged (1 kg per bag) in 15-cm x 22-cm FreshPak 500TM plastic bags (Koch Supplies Inc., Kansas City, MO) using a Multivac sealer (Multivac A300/22, Wolfertschwenden, Germany). All materials were stored at 4°C and analyzed within 24 hr of collection. Standard-flavor compounds were obtained from commercial sources such as Aldrich (Milwaukee, WI).

**Atmospheric simultaneous steam distillation-solvent extraction (A-SDE)**

Low quantities of volatile-flavor components in the claw meat and CPB made it necessary to use A-SDE, which yielded 10,000 to 20,000-fold concentrations of volatiles prior to analysis. A-SDE involved heating the samples; hence, formation of artifacts was inevitable. Nevertheless, since this experiment involved comparison of two cooked samples extracted under identical conditions, it was anticipated that interference from artifacts would occur to a similar extent in both samples, and such interference could indirectly reflect the amount of precursors that was present in each samples.

Extraction was performed on 500g claw meat or 1000g CPB using a Likens and Nickerson (1964) type A-SDE apparatus (Cat. No. K-523010-0000, Kontes, Vineland, NJ). Sample plus distilled water (1:2 [w/v]) and 100 μg of internal standard (2,4,6-trimethylpyridine) were extracted for 2 hr with 50 mL of redistilled dichloromethane as described by Tanchotikul and Hsieh (1991). Triplicate extractions were carried out for each sample. Extracts were concentrated to 0.05 mL using a gentle stream of nitrogen prior to the gas chromatographic (GC) analysis. Duplicate injections were made for each extract.

**Gas chromatography/mass spectrometry (GC/MS)**

The GC/MS system consisted of an HP 5790A GC, coupled with an HP 5970B mass-selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA). From each SDE extract, 5 μL were injected in the splitless mode (155°C injector; 30-sec valve delay) into a fused silica, open-tubular (FSOT) column (Supelcowax 10, 60 m length x 0.25 mm i.d. x 0.25 μm film thickness; Supelco, Inc., Bellefonte, PA). Oven temperature was programmed from 40°C to 195°C at a ramp rate of 2°C/min with the initial and final hold times of 5 and 40 min, respectively. The carrier gas, helium, was maintained at a linear velocity of 25 cm/s. MSD conditions were as follows: capillary direct MS interface temperature, 200°C; ion source temperature, 200°C; ionization voltage, 70 eV; mass range, 33-290 amu; and electron multiplier voltage, 2200 V. For most samples, the MSD was turned off twice from 3.5 to 4.5 min; and from 8.6 to 10.40 min during the 120-min acquisition time to avoid damage to the MSD due to excessive signals from some unknown compounds and from the solvent.

**Gas chromatography/Fourier transform infrared spectrometry (GC/FTIR)**

The GC/FTIR system consisted of an HP 5890 GC coupled with an HP 5965B infrared detector (IRD) (Hewlett-Packard Co., Palo Alto, CA). GC conditions were the same as above,

except separations were achieved on a wide-bore FSOT column (DB-WAX, 30 m length x 0.54 mm i.d. x 1.0 μm film thickness; J&W Scientific, Folsom, CA). The helium carrier-gas linear velocity was 30 cm/s. IRD conditions were as follows: light pipe and transfer lines both at 200°C; optical resolution, 8 $cm^{-1}$; and coadd factor, 2 scans/spectrum. Infrared radiation was detected by a narrow-band mercury cadmium telluride (MCT) detector inside the compartment of the IRD.

**Compound identification and relative abundance**

Peak identifications were based on comparison of GC-retention indices (RI) (van den Dool and Kratz, 1963) and mass and infrared spectra of unknowns with those of authentic-standard compounds under identical experimental conditions. The relative concentration of each peak was estimated by the ratio of total ion peak area with that of the internal standard. The relative abundance of coeluting components was estimated using mass chromatography (Hites and Biemann, 1970).

**Statistical analysis**

A two-tailed, two-sample t-statistic was used to analyze the statistical differences of each compound between the claw meat and the CPB (Moore and McCabe, 1989). Differences were considered significant when means

of compared sets differed at the $P<0.05$ level of significance.

A discussion with a statistician in the final stages of this dissertation research led to the conclusion that the t-statistic was improperly used because the underlying assumptions of the t-statistics were violated under the present experimental conditions (Pagano, R.R., 1986). Further, since the design of the present experiment involved two samples--claw and CPB-- collected at three different dates, and the analytical results involved a large number of chemical compounds (dependent variables), multivariate statistics, which take into considerations such multiple variables, should have been used. However, since the use of the t-statistic did not affect the results of the overall investigation, the data were not reanalyzed. Future work of this nature should use multivariate statistics.

## Results and Discussion

Typical total ion chromatograms of volatile components in fresh-picked claw meat and CPB (Fig. 3.1 and 3.2) show that combined totals of 98 compounds were identified in both samples. These included 77 compounds identified in claw meat and 80 in CPB (Table 3.1). Many compounds (54) were identified for the first time in blue crab, including seven aldehydes, 11 alkanes, five aromatic

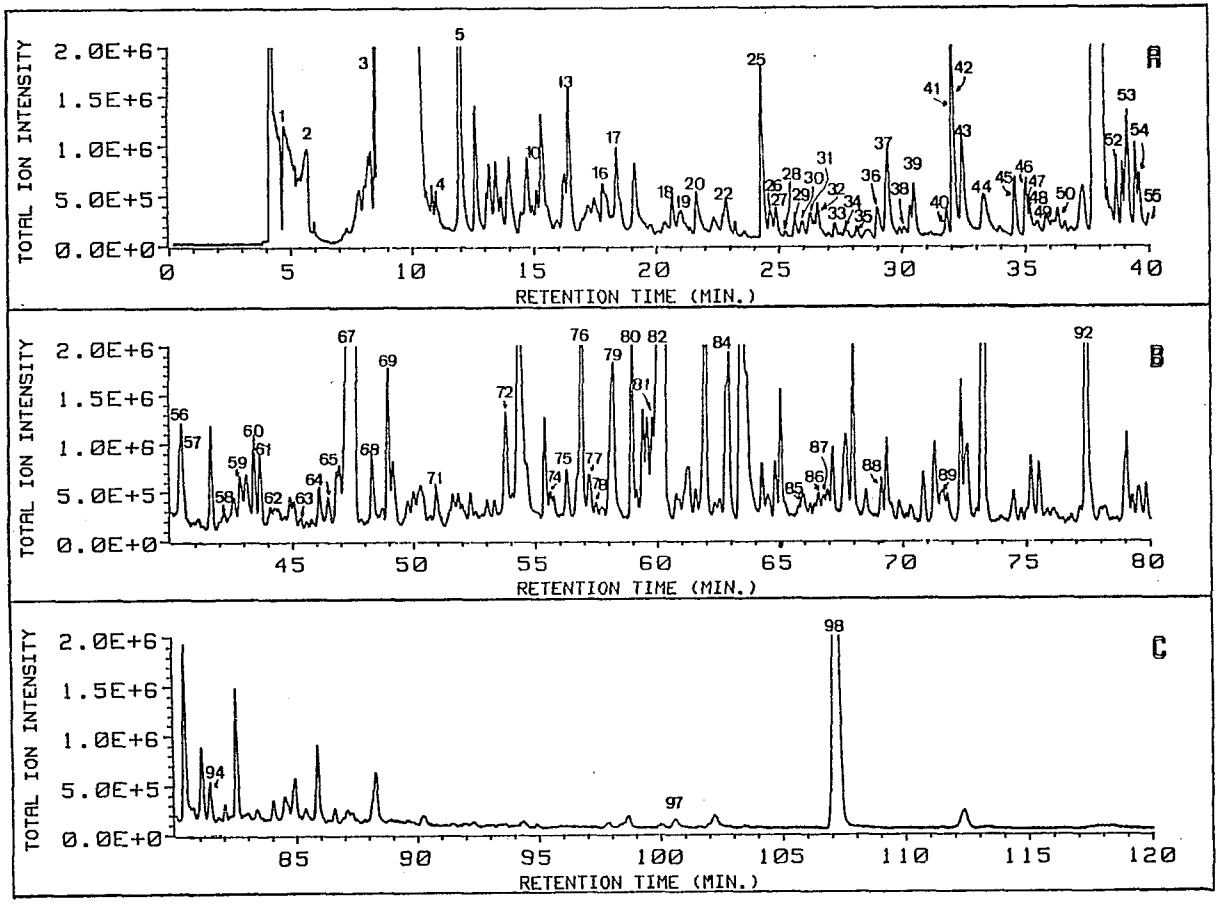



Figure 3.1 Total ion chromatogram of volatile components in blue crab claw meat between retention times of: (A) 0 to 40 min, (B) 40 to 80 min, and (C) 80 to 120 min. (Peak numbers correspond to those in Table 3.1)

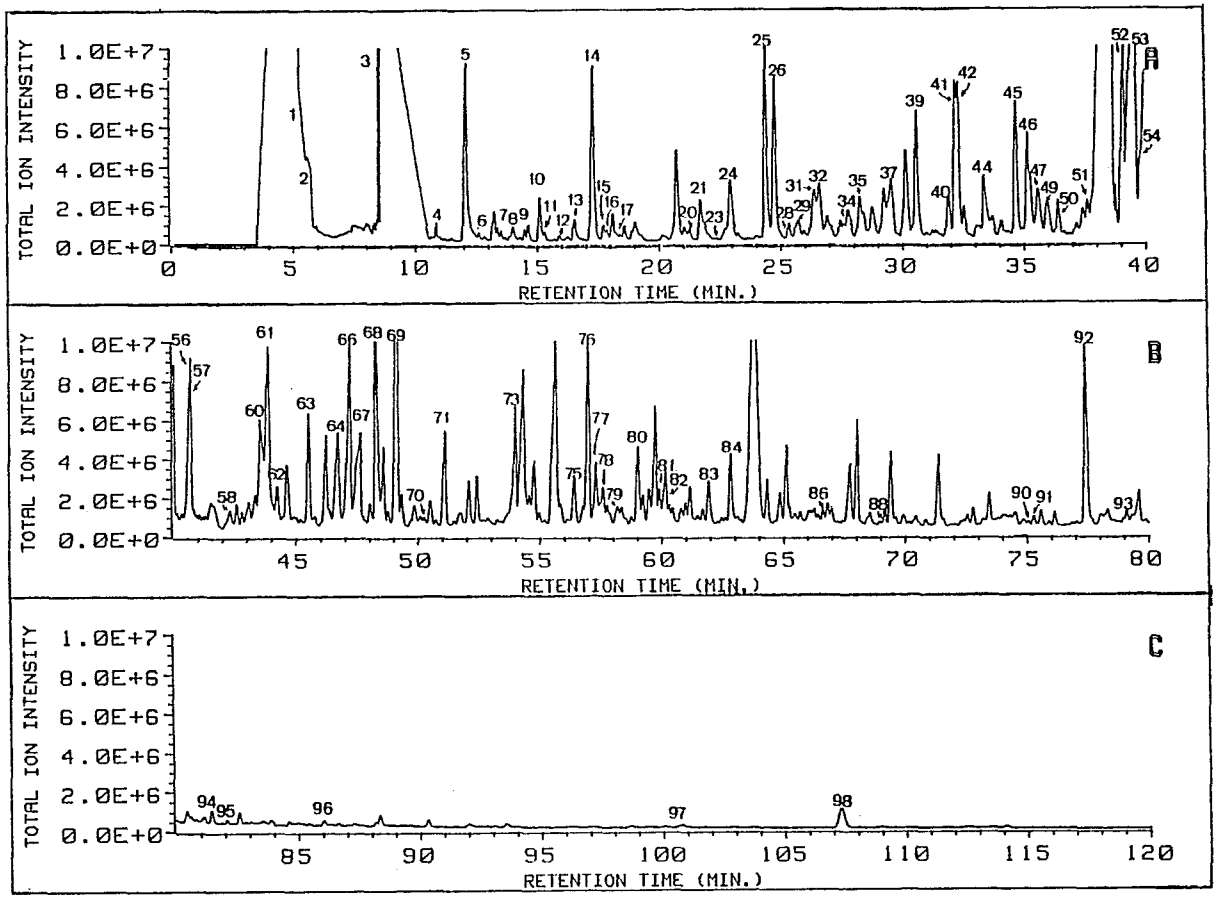



Figure 3.2 Total ion chromatogram of volatile components in blue crab processing by-product between retention times of: (A) 0 to 40 min, (B) 40 to 80 min, and (C) 80 to 120 min. (Peak numbers correspond to those in Table 3.1)

Table 3.1 Volatile compounds identified in fresh blue crab claw meat and/or processing by-product

| Peak no. | Compound | RI[1] | Ref[2] | Det.[3] Methods | Claw Meat Mean[4] ng/g | n[5] | RSD[6] (%) | Min[7] ng/g | Max[8] ng/g | By-Product Mean ng/g | n | RSD (%) | Min ng/g | Max ng/g |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Aldehydes** | | | | | | | | | | | | | |
| 11 | 2-butenal | 1050 | | RI,MS | | | | | | 1.2 | 12 | 35 | 1.0 | 1.3 |
| 16 | hexanal | 1080 | 1,3 | RI,MS | 2.1 | 14 | 74 | 0.48 | 3.1 | 1.3 | 12 | 69 | 0.78 | 1.74 |
| 23 | 2-methyl-2-pentenal | 1150 | | RI,MS | | | | | | 1.6 | 12 | 142 | 0.066 | 4.5 |
| 27 | heptanal | 1183 | 1,3 | RI,MS | 1.2 | 14 | 66 | 0.23 | 1.5 | | | | | |
| 33 | (E)-2-hexenal | 1216 | | RI,MS | 0.85 | 14 | 52 | 0.66 | 1.3 | | | | | |
| 36 | (Z)-4-heptenal | 1237 | | RI,MS | 1.5 | 14 | 54 | 1.2 | 1.6 | | | | | |
| 43 | octanal | 1290 | 3 | RI,MS | 3.5 | 14 | 68 | 2.5 | 4.3 | | | | | |
| 55 | nonanal | 1396 | | RI,MS | 0.86 | 14 | 109 | 0 | 1.2 | | | | | |
| 65 | (E,E)-2,4-heptadienal | 1496 | | RI,MS | 4.4 | 14 | 43 | 3.9 | 4.8 | | | | | |
| 69 | benzaldehyde* | 1527 | 2,3 | RI,MS,IR | 8.9 | 14 | 54 | 6.4 | 15 | 23 | 12 | 42 | 15 | 28 |
| 87 | (E,E)-2,4-decadienal | 1817 | | RI,MS | 2.7 | 14 | 52 | 1.8 | 4.0 | | | | | |
| | **Alkanes** | | | | | | | | | | | | | |
| 4 | 2,2,4,6,6-penta-methylheptane | 957 | | RI,MS | 1.6 | 14 | 53 | 1.3 | 2.1 | 0.52 | 12 | 59 | 0.23 | 0.77 |
| 6 | decane | 1000 | 1 | RI,MS | | | | | | 0.50 | 12 | 64 | 0.35 | 0.66 |
| 30 | dodecane | 1202 | | RI,MS | 0.71 | 14 | 40 | 0.65 | 0.86 | | | | | |
| 44 | tridecane | 1305 | | RI,MS | 26 | 14 | 52 | 20 | 36 | 35 | 12 | 70 | 6.9 | 53 |
| 56 | tetradecane | 1402 | | RI,MS | 19 | 14 | 61 | 9.2 | 30 | 20 | 12 | 17 | 18 | 23 |
| 67 | pentadecane* | 1500 | | RI,MS | 580 | 14 | 33 | 460 | 729 | 180 | 12 | 45 | 92 | 260 |
| 72 | hexadecane | 1600 | | RI,MS | 100 | 14 | 38 | 70 | 129 | | | | | |
| 79 | 2,6,10,14-tetramethyl-pentadecane* | 1672 | | RI,MS | 110 | 14 | 46 | 78 | 152 | 36 | 12 | 33 | 24 | 45 |
| 82 | heptadecane* | 1700 | | RI,MS,IR | 490 | 14 | 59 | 290 | 720 | 97 | 12 | 29 | 75 | 130 |
| 85 | octadecane | 1802 | | RI,MS | 34 | 14 | 43 | 30 | 38 | | | | | |
| 89 | nonadecane | 1900 | | RI,MS | 28 | 14 | 59 | 11 | 37 | | | | | |
| 95 | heneicosane | 2100 | | RI,MS | | | | | | 5.8 | 12 | 62 | 2.6 | 8.7 |

(table con'd.)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Aromatics** | | | | | | | | | | | | |
| 10 | methylbenzene | 1037 | 1,3 | RI,MS | 2.1 | 14 | 94 | 1.1 | 4.7 | 2.7 | 12 | 46 | 1.9 | 3.7 |
| 19 | ethylbenzene | 1123 | 1,3 | RI,MS | 0.91 | 14 | 78 | 0.49 | 1.8 | | | | | |
| 20 | p-xylene | 1136 | 1,3 | RI,MS | 2.4 | 14 | 120 | 0.61 | 6.3 | 2.4 | 12 | 68 | 0.40 | 3.4 |
| 21 | m-xylene | 1141 | 1,3 | RI,MS | | | | | | 2.6 | 12 | 110 | 0.66 | 6.2 |
| 34 | 1-ethyl-3-methylbenzene | 1223 | | RI,MS | 1.1 | 14 | 50 | 0.71 | 1.6 | 2.0 | 12 | 40 | 1.8 | 2.4 |
| 38 | styrene | 1256 | 1 | RI,MS | 0.87 | 14 | 86 | 0.44 | 1.8 | | | | | |
| 40 | 1,2,4-trimethylbenzene* | 1281 | 1,3 | RI,MS | 1.4 | 14 | 48 | 0.98 | 2.1 | 3.2 | 12 | 23 | 2.6 | 3.9 |
| 49 | 1,2,3-trimethylbenzene* | 1339 | | RI,MS | 1.3 | 14 | 55 | 0.87 | 2.2 | 3.4 | 12 | 22 | 2.6 | 3.7 |
| 58 | 1,2,4,5-tetramethylbenzene* | 1432 | | RI,MS | 1.4 | 14 | 63 | 1.1 | 2.0 | 3.0 | 12 | 16 | 2.7 | 3.3 |
| 60 | 1,3-dichlorobenzene* | 1446 | | RI,MS | 4.2 | 14 | 38 | 3.8 | 5.0 | 7.8 | 12 | 63 | 5.5 | 11 |
| 84 | naphthalene | 1749 | 1 | RI,MS | 14 | 14 | 54 | 4.7 | 18 | 9.9 | 12 | 35 | 5.4 | 13 |
| 92 | phenol | 2008 | 2,3 | RI,MS,IR | 25 | 14 | 52 | 8.4 | 40 | 19 | 12 | 43 | 8.9 | 25 |
| 94 | 4-methylphenol | 2087 | | RI,MS | 8.1 | 14 | 39 | 4.1 | 10 | 6.2 | 12 | 44 | 2.9 | 8.7 |
| | **Alcohols** | | | | | | | | | | | | | |
| 9 | 2-butanol | 1034 | | RI,MS | | | | | | 0.95 | 12 | 86 | 0.36 | 1.9 |
| 24 | 1-penten-3-ol | 1159 | | RI,MS | | | | | | 14 | 12 | 74 | 6.9 | 27 |
| 31 | 3-methyl-1-butanol | 1207 | | RI,MS | 2.5 | 14 | 78 | 1.4 | 4.9 | 4.4 | 12 | 25 | 4.2 | 4.8 |
| 37 | 1-pentanol | 1246 | 3 | RI,MS | 0.64 | 14 | 34 | 0.57 | 0.74 | 0.68 | 12 | 34 | 0.50 | 0.83 |
| 51 | 1-hexanol | 1358 | 3 | RI,MS | | | | | | 8.1 | 12 | 68 | 1.5 | 13 |
| 61 | 1-octen-3-ol* | 1449 | | RI,MS | 4.0 | 14 | 61 | 2.2 | 4.9 | 24 | 12 | 48 | 18 | 35 |
| 62 | 1-heptanol* | 1455 | 4 | RI,MS | 2.7 | 14 | 86 | 1.9 | 3.9 | 5.7 | 12 | 30 | 4.9 | 6.1 |
| 64 | 2-ethyl-1-hexanol* | 1489 | 4 | RI,MS | 2.2 | 14 | 48 | 1.5 | 3.2 | 6.2 | 12 | 35 | 3.9 | 8.3 |
| 71 | 1-octanol* | 1662 | | RI,MS,IR | 2.7 | 14 | 48 | 2.0 | 3.1 | 11 | 12 | 20 | 9.9 | 14 |
| 78 | 1-nonanol* | 1668 | | RI,MS,IR | 2.4 | 14 | 41 | 1.9 | 3.2 | 7.2 | 12 | 20 | 5.8 | 8.1 |
| 83 | 2-undecanol | 1726 | | RI,MS,IR | | | | | | 13 | 12 | 49 | 5.6 | 19 |
| 91 | 1-dodecanol | 1975 | 4 | RI,MS,IR | | | | | | 82 | 12 | 49 | 48 | 129 |
| 93 | nerolidol isomer | 2044 | | RI,MS | | | | | | 0.30 | 12 | 38 | 0.26 | 0.32 |
| 96 | 1-tetradecanol | 2173 | 4 | RI,MS | | | | | | 47 | 12 | 51 | 32 | 67 |
| 97 | 1-hexadecanol | 2381 | 4 | RI,MS,IR | 43 | 14 | 29 | 38 | 53 | 33 | 12 | 49 | 20 | 46 |
| | **Ketones** | | | | | | | | | | | | | |
| 3 | 2-propanone | 813 | 5 | RI,MS | 8.3 | 14 | 65 | 5.5 | 10 | 13 | 12 | 70 | 6.7 | 21 |
| 5 | 2,3-butanedione | 979 | | RI,MS | 30 | 14 | 33 | 25 | 33 | 29 | 12 | 35 | 29 | 30 |
| 7 | 4-methyl-2-pentanone | 1012 | 1 | RI,MS | | | | | | 2.1 | 12 | 82 | 0.61 | 3.3 |

(table con'd.)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Ketones** | | | | | | | | | | | | |
| 12 | 3-hexanone | 1053 | 1,3 | RI,MS | | | | | | 0.45 | 12 | 30 | 0.39 | 0.50 |
| 13 | 2,3-pentanedione* | 1058 | | RI,MS | 16 | 14 | 58 | 11 | 23 | 5.4 | 12 | 29 | 4.7 | 6.6 |
| 15 | 2-hexanone | 1083 | 1,3 | RI,MS | | | | | | 1.1 | 12 | 45 | 0.70 | 1.7 |
| 18 | (E)-3-penten-2-one | 1122 | | RI,MS | 0.81 | 14 | 84 | 0.46 | 1.7 | | | | | |
| 26 | 2-heptanone* | 1179 | 3 | RI,MS | 1.4 | 14 | 76 | 0.88 | 2.7 | 8.1 | 12 | 72 | 1.0 | 14 |
| 41 | 3-hydroxy-2-butanone* | 1284 | | RI,MS | 16 | 14 | 37 | 11 | 22 | 10 | 12 | 20 | 8.8 | 11 |
| 42 | 2-octanone* | 1284 | 3 | RI,MS | 0.40 | 14 | 46 | 0.28 | 0.68 | 8.4 | 12 | 27 | 6.3 | 9.5 |
| 54 | 2-nonanone* | 1391 | | RI,MS,IR | 1.9 | 14 | 66 | 1.1 | 3.2 | 14 | 12 | 17 | 12 | 16 |
| 59 | 2-cyclohexen-1-one | 1437 | | RI,MS | 4.4 | 14 | 52 | 2.4 | 6.8 | | | | | |
| 66 | 2-decanone | 1498 | | RI,MS | | | | | | 20 | 12 | 48 | 10 | 32 |
| 73 | 2-undecanone | 1609 | | RI,MS,IR | | | | | | 24 | 12 | 29 | 17 | 27 |
| 77 | acetophenone | 1655 | | RI,MS | 4.8 | 14 | 43 | 3.5 | 6.5 | 7.4 | 12 | 46 | 3.4 | 11 |
| 86 | 2-tridecanone | 1814 | | RI,MS | 26 | 14 | 38 | 14 | 32 | 38 | 12 | 29 | 34 | 47 |
| | **Pyrazines** | | | | | | | | | | | | | |
| 32 | pyrazine* | 1210 | 2 | RI,MS | 3.3 | 14 | 28 | 2.9 | 3.6 | 6.6 | 12 | 33 | 5.6 | 7.7 |
| 39 | 2-methylpyrazine* | 1261 | 2 | RI,MS | 3.3 | 14 | 53 | 2.5 | 4.7 | 11 | 12 | 20 | 8.8 | 12 |
| 45 | 2,5-dimethylpyrazine* | 1319 | 2 | RI,MS | 2.6 | 14 | 45 | 2.1 | 3.7 | 9.6 | 12 | 17 | 9.4 | 9.8 |
| 46 | 2,6-dimethylpyrazine* | 1326 | 2 | RI,MS | 1.9 | 14 | 37 | 1.7 | 2.2 | 5.7 | 12 | 39 | 4.0 | 7.0 |
| 48 | ethyl pyrazine | 1332 | | RI,MS | 1.4 | 14 | 52 | 0.87 | 2.2 | | | | | |
| 50 | 2,3-dimethylpyrazine* | 1344 | 2 | RI,MS | 1.9 | 14 | 69 | 1.7 | 2.3 | 2.9 | 12 | 16 | 2.6 | 3.1 |
| 57 | 2,3,5-trimethylpyrazine* | 1411 | 2 | RI,MS | 2.3 | 14 | 87 | 0 | 4.2 | 15 | 12 | 19 | 14 | 17 |
| 63 | tetramethylpyrazine* | 1474 | 2 | RI,MS | 0.62 | 14 | 73 | 0 | 1.0 | 10 | 12 | 33 | 10 | 10 |
| 74 | 2-acetylpyrazine | 1629 | | RI,MS | 1.8 | 14 | 83 | 0 | 3.7 | | | | | |
| | **S-containing compounds** | | | | | | | | | | | | | |
| 2 | carbon disulfide | ---- | | RT,MS | 48 | 14 | 59 | 30 | 61 | 84 | 12 | 32 | 63 | 101 |
| 14 | dimethyldisulfide | 1076 | 1,2,3 | RI,MS | | | | | | 21 | 12 | 105 | 4.9 | 48 |
| 17 | 2-methylthiophene* | 1090 | 1,3 | RI,MS | 4.0 | 14 | 42 | 3.5 | 4.7 | 0.61 | 12 | 64 | 0.38 | 0.98 |
| 52 | 2,4,5-trimethylthiazole | 1377 | | RI,MS | 6.8 | 14 | 59 | 3.0 | 9.0 | 11 | 12 | 39 | 6.5 | 16 |
| 53 | dimethyltrisulfide* | 1382 | 2 | RI,MS | 7.7 | 14 | 68 | 5.5 | 9.8 | 64 | 12 | 86 | 9.3 | 124 |
| 76 | 2-acetythiazole | 1650 | 2 | RI,MS,IR | 17 | 14 | 38 | 15 | 19 | 32 | 12 | 16 | 20 | 26 |
| 80 | 3-thiophenecarboxaldehyde | 1684 | | RI,MS | 12 | 14 | 34 | 8.9 | 13 | 9.0 | 12 | 55 | 3.0 | 13 |
| 90 | benzothiazole | 1970 | | RI,MS | | | | | | 1.7 | 12 | 38 | 1.3 | 2.1 |

(table con'd.)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Terpenes** | | | | | | | | | | | | |
| 29 | limonene | 1195 | 1,3 | RI,MS | 3.2 | 14 | 150 | 0.40 | 10 | 4.2 | 12 | 85 | 1.2 | 8.8 |
| 70 | linalool | 1554 | | RI,MS | | | | | | 2.1 | 12 | 22 | 1.8 | 2.4 |
| 75 | menthol | 1644 | | RI,MS,IR | 11 | 14 | 65 | 5.2 | 21 | 10 | 12 | 42 | 8.1 | 12 |
| 81 | alpha-terpineol | 1698 | | RI,MS | 10 | 14 | 25 | 8.8 | 12 | 11 | 12 | 36 | 7.3 | 15 |
| | **Miscellaneous compounds** | | | | | | | | | | | | | |
| 1 | trimethylamine | ---- | 5 | RT,MS | 230 | 2 | 8.5 | -[9] | - | 174 | 2 | 15 | - | - |
| 8 | chloroform | 1022 | | RI,MS | | | | | | 2.9 | 12 | 65 | 1.5 | 5.1 |
| 22 | 7-oxabicyclo[4.1.0]heptane | 1152 | | RI,MS | 0.44 | 14 | 69 | 0.30 | 0.66 | | | | | |
| 25 | pyridine | 1174 | 2 | RI,MS | 8.7 | 14 | 57 | 5.5 | 15 | 17 | 12 | 23 | 14 | 19 |
| 28 | trimethyloxazole* | 1188 | 2 | RI,MS | 0.75 | 14 | 48 | 0.64 | 0.80 | 5.4 | 12 | 129 | 0.61 | 14 |
| 35 | 2-pentylfuran* | 1229 | 1,3 | RI,MS | 0.43 | 14 | 40 | 0.36 | 0.52 | 6.0 | 12 | 80 | 2.7 | 12 |
| 47 | N,N-dimethylformamide | 1328 | | RI,MS,IR | 3.5 | 14 | 65 | 1.8 | 5.2 | 5.3 | 12 | 59 | 2.2 | 8.5 |
| 68 | 1H-pyrrole* | 1514 | 1,2 | RI,MS | 8.2 | 14 | 69 | 4.5 | 16 | 25 | 12 | 17 | 25 | 25 |
| 88 | geranylacetone | 1859 | | RI,MS | 48 | 14 | 40 | 23 | 60 | 61 | 12 | 32 | 52 | 74 |
| 98 | indole* | 2444 | 2 | RI,MS,IR | 86 | 14 | 96 | 24 | 139 | 39 | 12 | 49 | 28 | 61 |

[1]: retention index; ----: retention index was not determined.
[2]: compound previously identified in the following reference(s): 1-Matiella and Hsieh, 1990; 2-Ando and Osawa, 1988; 3-Hsieh et al., 1989; 4-Flament, 1990; 5-Rayner et al., 1981.
[3]: MS: mass spectrum; IR: infrared spectrum; RI: retention index; RT: retention time.
[4]: arithmatic mean concentration of a volatile compound based on the number of detected peaks.
[5]: number of detected peaks.
[6]: relative standard deviation.
[7]: minimum relative mean concentration of a volatile compound detected in 3 sampling days.
[8]: maximum relative mean concentration of a volatile compound detected in 3 sampling days.
[9]: -: minimum and maximum relative mean concentrations were not determined.
*: mean values are significantly different ($P<0.05$).

compounds, four miscellaneous compounds, eight alcohols, 10 ketones, two pyrazines, four sulfur-containing compounds, and three terpenes. The average concentration ranges of each compound and relative standard deviations (RSD) from the three collection days were compared (Table 3.1). Most compounds had RSD values close to 50%; however, several had extremely high RSD values (>100%), e.g., p-xylene, m-xylene. Such high values indicate large natural fluctuations in the concentrations of these compounds. The calculations were based on the concentration of each detected peak in all the chromatograms. The possible number of detected peaks was 18. However, due to the error of over-concentration of some extracts, their analytical results were not considered in the calculation of mean concentrations. Since the maximum number of detected peaks was 14 and 12 for claw meat and CPB, respectively, differences in the number of detected peaks appeared. Compounds that were too low in concentration to produce any detectable values during instrumental analysis were assigned a value of zero so that the resulting number of detected peaks would agree with the maximum possible number in each sample. Among the 58 common compounds found in both samples, the statistical mean concentrations of 24 compounds were higher in CPB, while seven compounds were higher in claw meat than in the other sample.

**Aldehydes**

Nine aldehydes were found in claw meat, whereas only four were found in CPB. Only hexanal and benzaldehyde were present in both samples. Compounds such as (E)-2-hexenal, (Z)-4-heptenal, nonanal, (E,E)-2,4-heptadienal, and (E,E)-2,4-decadienal were identified for the first time in blue crab and were detected only in claw meat. The majority of those identified aldehydes were considered lipid autoxidation products. Some aldehydes may be detrimental to crab aroma because they may mask the desirable aroma of cooked crab, e.g. aroma of 2,4,7-decatrienal isomers have been described as burnt/fishy (Meijboom and Stroink, 1972). Statistically higher mean concentrations of benzaldehyde were found in CPB (23 ng/g) compared with the claw meat (8.9 ng/g). Benzaldehyde may have a desirable effect on claw meat aroma since it has a pleasant almond, nutty, fruity aroma (Vejaphan et al., 1988).

**Alkanes**

With the exception of decane, all alkanes in Table 3.1 are reported for the first time in blue crab. Statistical mean concentrations of those alkanes detected in both samples were generally higher in claw meat than in CPB. The total concentration of all alkanes in claw meat was 3.7 times higher than that in CPB. Alkanes are

believed to contribute very little to the overall flavor of foods (Grosch, 1982). However, there may be some notable exceptions, especially among the branched-chain alkanes. For example, 2,6,10,14-tetramethylpentadecane, which was significantly higher in the claw meat than in CPB, was reported to contribute a grass-like (green), sweet aroma to crayfish processing by-product (Tanchotikul and Hsieh, 1989).

**Aromatic compounds**

The presence of alkylbenzenes has been reported in shrimp, crab, and crayfish (Shye et al., 1987; Tanchotikul and Hsieh, 1989; Matiella and Hsieh, 1990). Three alkylbenzenes (1,2,4-trimethylbenzene, 1,2,3-trimethylbenzene, and 1,2,4,5-tetramethylbenzene) and 1,3-dichlorobenzene were higher in statistical mean concentrations in CPB than in claw meat. Compounds 1,2,4-trimethylbenzene and 1,2,4,5-tetramethylbenzene together produced a naphthalene-like note in roast beef fat. Besides, the former retained a slight green aroma and the latter a slight floral aroma (Min et al., 1977). The mean concentrations of both phenol and 4-methylphenol were high compared with other aromatic compounds. Phenol and 4-methylphenol contributed phenolic "sheepy" aroma and "piggy" aroma, respectively, in cattle (Ha and Lindsay, 1991).

**Alcohols**

Eight alcohols were detected common to both claw meat and CPB. Among them, only the statistical mean concentrations of 1-octen-3-ol, 2-ethyl-1-hexanol, 1-heptanol, 1-octanol, and 1-nonanol were significantly higher in CPB than in claw meat. More high-molecular-weight alcohols (>C10) were found in CPB than in claw meat. Such alcohols could originate from lipids. Flament (1990) detected 19 alcohols in the neutral fraction of a crab extract powder, most being described as having sweet, floral, and fruity aromas.

**Ketones**

Eleven ketones were found in claw meat and 14 in CPB. Nine ketones were detected in both. Ten ketones are reported for the first time in blue crab, including 2,3-butanedione, 2,3-pentanedione, (E)-3-penten-2-one, 3-hydroxy-2-butanone, 2-nonanone, 2-cyclohexen-1-one, 2-decanone, 2-undecanone, acetophenone, and 2-tridecanone. The compound 2,3-butanedione had a characteristic buttery note and has been identified in cooked beef and crayfish (Chang and Peterson, 1977; Tanchotikul and Hsieh, 1989). Statistically higher mean concentrations of 2,3-pentanedione and 3-hydroxy-2-butanone were detected in claw meat than in CPB, while statistically higher levels of 2-heptanone, 2-octanone, and 2-nonanone were found in

CPB than in claw meat. No significant differences were found between the claw meat and CPB with regards to the statistical mean concentrations of 2-propanone, 2,3-butanedione, acetophenone, and 2-tridecanone.

**Pyrazines**

A combined total of nine pyrazines was found in claw meat and CPB. Two pyrazines, ethylpyrazine and 2-acetylpyrazine, found only in the claw meat, were reported in blue crab for the first time. The rest have been previously reported in crab (Ando and Osawa, 1988). Though claw meat contained different types of pyrazine, it did not contain statistically higher mean concentrations of them than were found in CPB. Pyrazines, having low flavor thresholds, generally contribute to the desirable nutty, roasted flavors in cooked foods and beverages (Shibamoto, 1989) and are probably important to the flavor of cooked crab.

**Sulfur-containing compounds**

Six sulfur-containing compounds were found common in both claw meat and CPB. Among them, only dimethyldisulfide and benzothiazole were detected in CPB. The mean concentration of 2-methylthiophene was significantly higher in claw meat than in CPB. The statistical mean

concentration of dimethyltrisulfide was about eight times higher in CPB than in claw meat.

Aroma quality of CPB may be severely affected because of the combined high mean concentrations of both dimethyltrisulfide and dimethyldisulfide. Low mean concentrations of both compounds are necessary and desirable in some foods, such as chives and onions (Kallio et al., 1990). Tanchotikul and Hsieh (1989) described dimethyltrisulfide in crayfish by-product as having a green and vegetable-like aroma. The aroma of dimethyldisulfide has been described by various researchers as onion-, cabbage-, sulfur- and bad egg-like (MacLeod and Cave, 1976; Fors, 1983; Vejaphan et al., 1988).

**Terpenes**

Limonene, α-terpineol, and menthol were found at similar mean concentration levels in both claw meat and in CPB. Linalool was found only in CPB. These compounds probably originated from the diet of crab. While these terpenes all have unique and desirable aromas, they probably do not significantly affect the flavor of crab.

**Miscellaneous Compounds**

Claw meat contained a significantly higher mean concentration of indole in claw meat (86.4 ng/g) than in

CPB (39.0 ng/g). The odor of indole has been described as undesirable and naphthalene-like (Dravnieks, 1985).

The mean concentration of trimethylamine (TMA) was high compared with other compounds cited in Table 3.1. The mean concentration of TMA in each sample (claw meat and CPB) was based on the results from duplicate injections of one extract from one sampling day, therefore, a total of two occurances of TMA peaks was possible in the claw and in the CPB. This was because the analytical instrument--mass spectrometer--was purposely set off to avoid the detection of highly concentrated components such as the solvent and other unknown components at the beginning of the data acquisition. Even though the mean concentrations of TMA in both claw meat and CPB were statistically the same, the results could not be generalized to other extracts because the sample size for the analysis was too small. Josephson and Lindsay (1986) considered TMA to be an important contributor to overall boiled crab-type aroma.

Mean concentrations of trimethyloxazole, 2-pentylfuran, and 1H-pyrrole were significantly higher in the CPB than in claw meat. Matiella and Hsieh (1990) reported the presence of 2-pentylfuran in both boiled and pasteurized blue crab crabmeat samples. This compound was described as sweet, spicy, and green in crayfish processing by-product (Tanchotikul and Hsieh, 1989).

However, it introduced a beany, grassy note to the reversion flavor of soybean oil (Krishnamurthy et al., 1967). Compounds like 1H-pyrrole had a nutty flavor in crayfish by-product and a sweet and slightly burnt note in boiled crayfish tail meat (Vejaphan et al., 1988; Tanchotikul and Hsieh, 1989).

**Conclusions**

Not all 77 volatile compounds detected in claw meat necessarily contribute favorably to claw meat aroma. High concentrations of dimethyldisulfide and dimethyltrisulfide might have detrimental effects on overall aroma quality by masking desirable aromas. Whitfield et al. (1981) reported that the microbial spoilage of raw royal red prawn produced dimethyltrisulfide, which caused an off-flavor. Similar microbial spoilage might occur with injured or dead crabs during transportation to processing facilities. Spoiled crabs are generally discarded into the same collecting site (e.g., bin) during picking operations. Such a practice could produce CPB of low quality, since the spoiled crab may taint the aroma of the higher-quality CPB. Separation of deteriorated animals from other CPB would be crucial to ensure high-quality CPB for further processing.

All in all, among the combined total of 98 identified volatile compounds, 59 compounds were found in both claw

meat and CPB. Except aldehydes, more than half of the compounds in each group in claw meat could be identified in CPB. In fact, 77% of the compounds in claw could be found in CPB. Based on the these results, it is believed that CPB can be a potential source for flavor recovery.

# CHAPTER 4

# AROMA EXTRACT DILUTION ANALYSIS OF BLUE CRAB CLAW MEAT VOLATILE EXTRACTS PREPARED BY ATMOSPHERIC AND VACUUM SIMULTANEOUS STEAM DISTILLATION-SOLVENT EXTRACTION

## Introduction

The volatile flavor of cooked blue crab (*Callinectes sapidus*) has been investigated by various researchers (Rayner et al., 1981; Matiella and Hsieh, 1990; Chung and Cadwallader, 1993). However, compounds essential for authentic crab flavor have not been determined. One procedure used to establish predominant flavor compounds in foods is aroma extract dilution analysis (AEDA) (Gasser and Grosch, 1988; Blank and Grosch, 1991) in which a mixture of flavor compounds, or flavor extract, is separated by gas chromatography and the odor-activity of each component is evaluated by olfactometry. The value of odor-activity is dimensionless but is expressed as flavor dilution (FD)-factor in AEDA, which is defined as "the ratio of the concentration of a compound in the initial extract to that in the most diluted extract in which the odor was detected by gas chromatography/olfactometry (GC/O)" (Blank and Grosch, 1991). FD-factor is used to compare intensities of odor-active compounds in an extract. The objective of the present study was to

identify and compare the predominant volatile flavor compounds in cooked crab claw meat by atmospheric (A-SDE) and vacuum simultaneous steam distillation-solvent extraction (V-SDE) and AEDA.

## Materials and Methods

### Materials

A total of 5 lb freshly cooked blue crab claw meat was purchased in the summer of 1992 from two seafood outlets in Baton Rouge, Louisiana. At the outlets, original samples were kept in ice in a refrigerated shelf. All samples in 1-lb plastic containers were transported on ice to the LSU Food Science Department and stored (<24 hr) at 4°C until extracted.

### Atmospheric simultaneous steam distillation-solvent extraction (A-SDE)

Five hundred grams of sample plus distilled water [1:2 (w/v)] were extracted for two hours with redistilled dichloromethane (50 mL) in a Likens and Nickerson (1964) type A-SDE apparatus (Cat. No. K-523010-0000, Kontes, Vineland, NJ) as described by Tanchotikul and Hsieh (1989). Extracts were dried over 3 g of anhydrous sodium sulfate and concentrated under a stream of ultra-high-purity nitrogen to 1 mL. Duplicte extractions were performed.

**Vacuum simultaneous steam distillation-solvent extraction (V-SDE)**

The extraction procedure was similar to that described for A-SDE, except that an A-SDE apparatus modified for vacuum extraction was used. The modifications from the standard SDE apparatus were as follows: 1) vacuum valve replaced the U-joint valve; 2) a liquid-nitrogen cold trap and a vacuum valve were installed between V-SDE and vacuum pump; and 3) a 4-L, three-neck, round-bottom sample flask was modified with one neck for the vacuum valve and one for the thermometer. Vacuum and temperature were maintained at 30 in. Hg and 45-60°C, respectively. Extract was collected after two hours, concentrated under a stream of nitrogen gas to 10 mL, dried over 3 g of anhydrous sodium sulfate, and further reduced to 1 mL.

**Gas chromatography/mass spectrometry and flame ionization detection/olfactometry (GC/MS-FID/O)**

For qualitative analysis, an analytical system consisting of an HP 5790A GC coupled with both a flame ionization detector (FID) and an HP 5970B mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA) was used. Five $\mu$L of each extract were injected (splitless mode; 200°C injector temperature; 1-min valve delay) simultaneously into dual (closely matched) fused silica open tubular (FSOT) columns (Supelcowax 10, 60 m length x

0.25 mm i.d. x 0.25 μm film thickness; Supelco, Inc., Bellefonte, PA). Injector effluent, after passing through a FSOT precolumn (1 m length x 0.25 mm i.d. x 0.25 μm film thickness), was split 1:1 to each column using a glass Y-splitter. Column A was connected to the MSD, while the end of column B was split 1:1 to an FID and a sniffing port. Humidified air was mixed with the GC effluent in a transfer line near the outlet to the sniffing port. The temperatures of FID and sniffing port transfer line were 250 and 200°C, respectively. Helium was used as the carrier gas and maintained at a linear velocity of 25 cm/s. It was necessary to partially restrict the flow between column A and MSD (using ca. 1 m length x 0.1 mm i.d. FSOT column) in order to achieve the same linear velocity for both columns. Oven temperature was programmed from 40 to 195°C at a ramp rate of 2°C/min. The initial and final hold times were 5 and 40 min, respectively. MSD conditions were as follows: capillary direct MS interface temperature, 200°C; ion source temperature, 200°C; ionization voltage, 70 eV; mass range, 33-290 a.m.u.; scan rate, 1.67 scans/sec; and electron multiplier voltage, 1800 V.

**Aroma extract dilutions**

Serial dilutions were prepared from the 1.0 mL concentrated extracts in the ratio of 1:1 in

dichloromethane. Each dilution was transferred into separate 2-mL amber vials equipped with teflon-lined screw cap and stored at -80°C until analyzed.

**Gas chromatography/olfactometry (GC/O)**

The GC/O system consisted of an HP 5790A GC (Hewlett-Packard Co., Palo Alto, CA) equipped with a FSOT column (Supelcowax 10; 60 m x 0.32 mm i.d. x 0.25 $\mu$m film thickness; Supelco, Inc., Bellefonte, PA), an FID and a sniffing port. Effluent from the end of the GC column was split 1:1 between the FID and the sniffing port. Each dilution (5 $\mu$L) was injected in the splitless mode (200°C injector temperature; 1-min valve delay). Conditions of the GC oven and carrier gas were the same as described above. The FID temperature was kept at 250°C. GC/O was performed by two panelists familiar with crab flavor and olfactometric techniques.

**Data reduction**

The criterion in the reduction process of each detected aroma was based on the number of times it was detected in all the FD-chromatograms from the same extract. Each aroma note was counted based on its detection at the same retention index and with the same aroma attribute under the same GC/O conditions. In the present experiment, an aroma note with a count of two or

less from four FD-chromatograms was not considered significant and would be dropped from the final calculation of the average $Log_2$(FD-factor). The criterion was arbitrarily set since there is no standard technique in the literature. However, considering that the determination of threshold of a compound requires, 50% of the panelists recognizing the compound (ASTM, 1968), a similar criterion was adopted and applied to this experiment.

**Estimation of average FD-factor**

In order to summarize the GC/O results of each odorant, the average FD-factor of each flavor note was estimated by the arithmetic mean (N) of the $log_2$(FD-factor). (N) is readily converted back to FD-factor by:

(1) $$\text{FD-factor} = 2^N.$$

**Compound identification**

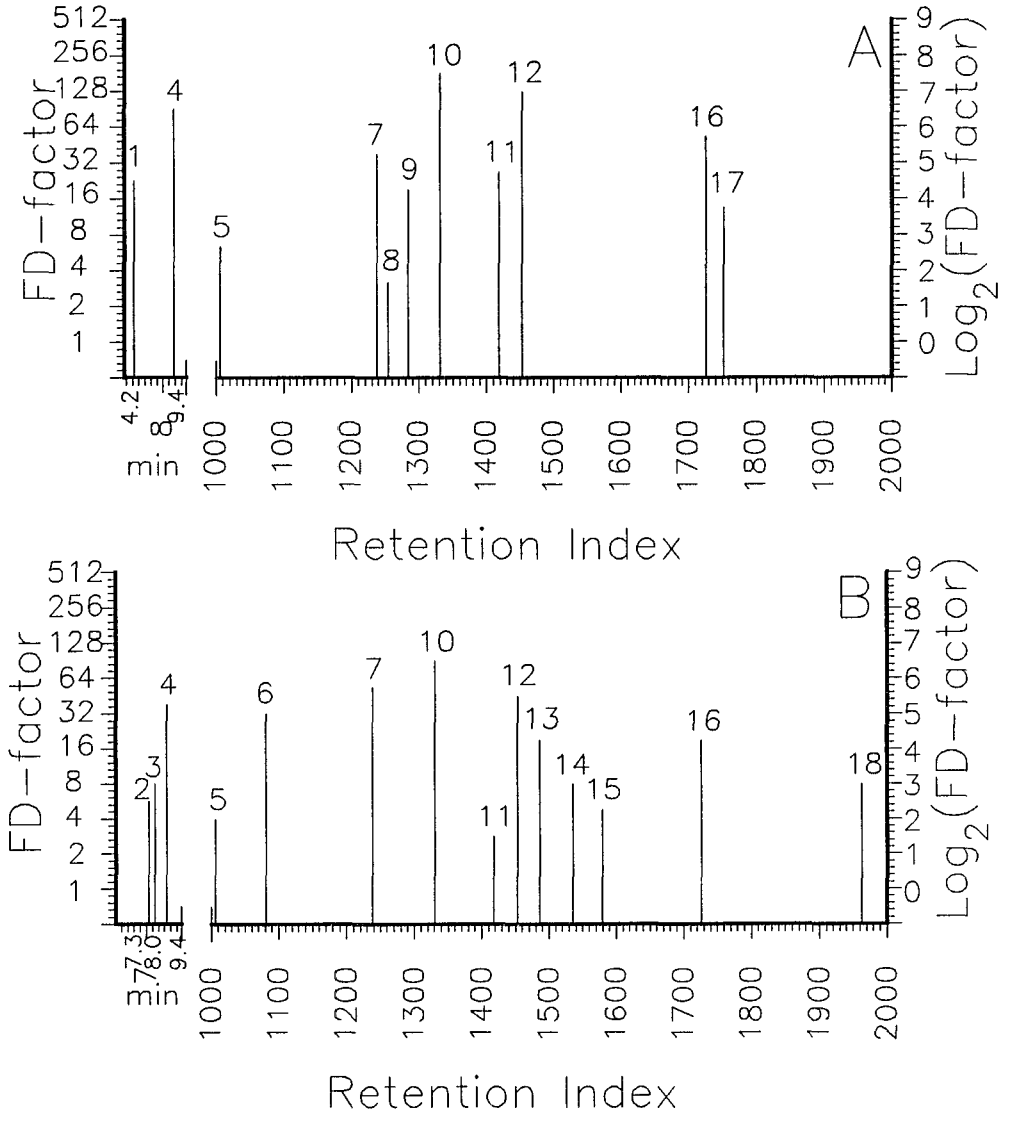
Identifications were based on comparison of GC retention indices (RI)(van den Dool and Kratz, 1963), mass spectra, and aroma properties of unknowns with those of authentic standard compounds analyzed under identical experimental conditions.

## Results and Discussion

### Comparison of A-SDE and V-SDE extracts

The average number of odor-active compounds detected in four original FD-chromatograms from A-SDE extracts of claw meat was 39 and ranged from 31 to 44 compounds, while an average number of 29 compounds, ranging from 18 to 41, was detected V-SDE extracts of claw meat. During both extractions, thermal generation of some artifacts was possible. Since higher temperatures were reached during A-SDE, this might promote the production of a wide variety of aroma compounds, thus resulting in a higher average number of detected aroma notes (39) compared with V-SDE (29). After data reduction, the number of odor-active compounds that represented a more consistent view of aroma notes isolated by A-SDE and V-SDE were 11 and 14, respectively. This seemed to reflect the randomness of the artifact generation, especially during A-SDE.

Since the original crab aroma was thermally generated during cooking, important and representative crab claw meat aroma notes were assumed to be stable and to exist in each extract. By comparing the FD-chromatograms of both extracts (Figure 4.3A and B), seven common aroma notes were found in both A-SDE and V-SDE extracts. They were described as creamy (no. 4); alkaline, raw egg-like (no. 5); potato-like (no. 7); nutty, popcorn-like (no. 10); nutty (no. 11); salty, soy sauce-like (no. 12);




| | | | | | |
|---|---|---|---|---|---|
| 1 | fishy | 7 | potato | 13 | moldy |
| 2 | natural gas | 8 | paint | 14 | potato |
| 3 | solvent | 9 | herbal | 15 | green |
| 4 | sour/diacetyl | 10 | popcorn/nutty | 16 | dry seaweed/fishy |
| 5 | alkaline/raw egg | 11 | nutty | 17 | fishy |
| 6 | burnt/sweet | 12 | salty/soy sauce | 18 | dry seaweed |

**Figure 4.3 Flavor dilution (FD)-chromatograms of blue crab claw meat volatiles isolated by: (A) Atmospheric simultaneous steam distillation-solvent extraction (A-SDE), and (B) Vacuum simultaneous steam distillation-solvent extraction (V-SDE).**

and dry seaweed-like, fishy (no. 16). Except for the potato-like note (no. 7), all other common notes had higher average FD-factors in A-SDE extracts than in the V-SDE extracts. The nutty, popcorn-like note (no. 10) had the highest average FD-factor in each average FD-chromatogram. Both extracts contained strong impressions of nutty and raw potato-like aromas mixed with dry seaweed-like aromas. Although Table 4.2 indicated that only a few potato-like and seaweed-like aromas were detected, the original FD-chromatograms (not shown) contained a substantial number. However, many of these were eliminated after data reduction.

**Identity of aroma notes**

Two compounds, identified as (Z)-4-heptenal (no. 7) and 2-acetyl-1-pyrroline (2-AP) (no. 10), had potato-like and popcorn-like aromas, respectively. Chung and Cadwallader (1993) identified (Z)-4-heptenal in freshly cooked crabmeat while none was found in CPB under the same A-SDE conditions. Some investigators have suggested the accumulation of (Z)-4-heptenal in certain foods (i.e., cod, soybean oil and alligator) was undesirable (Seals and Hammond, 1970; McGill et al., 1974; Cadwallader et al., 1994). However, its presence in freshly cooked crabmeat

Table 4.2 Odorous compounds detected in atmospheric and/or vacuum simultaneous steam distillation-solvent extracts of blue crab claw meat

| No.[1] | Aroma Description | RI/RT[2] | Average[3] $Log_2$(FD-factor) A-SDE | V-SDE | Compound |
|---|---|---|---|---|---|
| 1 | fishy | (4.2 min) | 5.50 | -[4] | trimethylamine |
| 2 | natural gas | (7.3 min) | - | 3.50 | unknown |
| 3 | solvent | (8.0 min) | - | 4.00 | unknown |
| 4 | sour/diacetyl | (9.4 min) | 7.50 | 6.25 | 2,3-butanedione |
| 5 | alkaline/raw egg | 1006 | 3.67 | 3.00 | pyrrolidine |
| 6 | burnt/sweet | 1081 | - | 6.00 | unknown |
| 7 | potato | 1238 | 6.25 | 6.75 | (Z)-4-heptenal |
| 8 | paint | 1255 | 2.67 | - | unknown |
| 9 | herbal | 1284 | 5.25 | - | unknown |
| 10 | popcorn/nutty | 1331 | 8.50 | 7.50 | 2-acetyl-1-pyrroline |
| 11 | nutty | 1418 | 5.75 | 2.50 | unknown |
| 12 | salty/soy sauce | 1453 | 8.00 | 6.50 | 3-(methylthio)-propanal |
| 13 | moldy | 1486 | - | 5.25 | unknown |
| 14 | potato | 1535 | - | 4.00 | (E)-4-decenal |
| 15 | green | 1579 | - | 3.25 | unknown |
| 16 | dry seaweed/fishy | 1726 | 6.75 | 5.25 | unknown |
| 17 | fishy | 1752 | 4.75 | - | unknown |
| 18 | dry seaweed | 1963 | - | 4.00 | unknown |

[1] Numbers correspond to those in the flavor dilution (FD)-chromatograms.
[2] RI=retention index; RT=retention time.
Numbers in parentheses represent average retention times.
[3] A-SDE: Atmospheric simultaneous steam distillation-solvent extract.
V-SDE: Vacuum simultaneous steam distillation-solvent extract.
[4] - : odorant was not detected in the extract.

might be desirable. McGill et al. (1974) described the aroma of (Z)-4-heptenal as being similar to boiled potatoes, and its threshold was determined to be 0.04 ppb.

For the first time, 2-AP was identified in blue crab. This compound has been associated with the popcorn, nutty aroma of scented rice (Buttery et al., 1983). These investigators suggested that 2-AP was the most potent compound among the "cracker-like" group of odor compounds and reported its odor threshold in water as 0.1 ppb. Comparison of the presence of 2-AP between raw and cooked scented rice by Buttery et al. (1983) suggested it was thermally generated during cooking. Griffith and Hammond (1989), in an attempt to generate Swiss cheese flavor by reacting amino acids and carbonyl compounds at room temperature, produced 2-AP from several mixtures, e.g. glyoxal and lysine. In the present study, 2-AP had the highest average FD-factor in both types of extracts, which seemed to reflect its importance to claw meat aroma.

The compound 2,3-butanedione was among the strongest notes detected in both extracts, with a threshold of 2.6 ppb in water (Fors, 1983). It is a characteristic product in cooked food and is thermally generated through the Maillard reaction (Hodge, 1967). Chung and Cadwallader (1993), using A-SDE, detected high average concentrations of 2,3-butanedione in both freshly cooked blue crabmeat and its CPB at 30 and 29 ng/g, respectively. However,

Hsieh et al. (1989) and Matiella and Hsieh (1990) did not detect 2,3-butanedione when using dynamic headspace sampling (DHS). The discrepancy might be due to the type as well as the conditions of the extraction procedures employed.

Pyrrolidine (no. 5), having an alkaline, raw egg-like aroma, was identified for the first time in blue crab; however, its aroma was among the weakest found in both samples. Its odor threshold is 0.15 ppm (Devos, 1990). Pyrrolidine has been detected in seawater (Yang et al., 1993) and can be formed biologically by bacteria, e.g. *Clostridium perfringens,* under low sugar conditions, as well as chemically by decarboxylation of proline via the Strecker degradation (Allison and MacFarlane, 1989; Griffith and Hammond, 1989). This compound was reported as a volatile component in whiskey and alfalfa (Viro, 1984; Srinivas, 1988). Recently, Okumura et al. (1993) proposed the use of pyrrolidine and other additives for prolonging the alkali and amine odor in Chinese noodles.

A salty, soy sauce-like aroma (no. 12), was detected which had a characteristic crabmeat quality. The compound responsible for this aroma was identified as 3-(methylthio)-propanal (3-MP) and its existence is being reported for the first time in blue crab. The compound 3-MP has a low odor threshold of 0.2 ppb (Guadagni et al.,

1972) and could be formed via the Maillard reaction using methionine as a precursor (Morton, 1960).

The compound (E)-4-decenal was detected in only V-SDE extracts and was described as potato-like. Although this compound contributed to the potato-like aroma in V-SDE extracts, its importance to the overall aroma of crabmeat is questionable since it was absent in the A-SDE extracts. Ames and Macleod (1984) detected (E)-4-decenal in unflavored textured soy protein and proposed that the compound originated from either lipid oxidation or lipid degradation. Hsu et al. (1982) also detected this compound in the neutral fraction of roasted beef.

Trimethylamine (TMA) has been described in the literature as ammonia-like and fishy (Dravnieks, 1985). This odorant was the earliest to elute during GC/O and was detected only in the A-SDE extracts. Since the volatility of TMA is high, extraction of it by V-SDE might not be as efficient as A-SDE. Chung and Cadwallader (1993) reported that similar amounts of TMA were found in the A-SDE of both fresh claw meat and CPB. Its odor threshold is 2.40 ppb (Devos et al., 1990). TMA is not present in fresh muscle but will increase during the postmortem bacterial reduction of trimethylamine oxide (TMAO) (Yamada, 1967). Blue crabs contain about 65 mg/100g of TMAO in their muscle (Yamada, 1967).

Two unidentified and seemingly predominant aromas were detected in both extracts and were described as nutty (RI=1418, no. 11) and dry seaweed-like (RI=1726, no. 16). These compounds could not be identified due to their low concentrations.

Based on the results of this study, the aroma or volatile flavor of blue crabmeat could be defined based principally on seven individual compounds. Many of these compounds are identified for the first time in crab claw meat, which seems to indicate that previous attempts by others, such as Hsieh et al. (1989), Matiella and Hsieh (1990), etc., at defining crab aroma failed to recognize those important compounds responsible for much of the characteristic crab claw meat aroma. Most of these compounds have extremely low threshold values and are more effectively detected by GC/O. Their low concentrations probably contributed to their lack of detection by other GC detectors.

# CHAPTER 5

# COMPARISON OF THE AROMA OF COOKED BLUE CRAB (*Callinectes sapidus*) CLAW AND LUMP MEATS

## Introduction

Crabmeat is sold in various forms in retail stores. In Louisiana, as well as in other states, most crab processors cook live crabs the day before the meat-picking activity commences, allowing the cooked crabs to cool overnight. After picking, crabmeat is generally divided according to the anatomical region from which it originates, e.g. claw, body (lump) and white meats. Crab connoisseurs have long claimed that the flavor of meat from various parts of the crab is different, yet little scientific evidence has been put forward to prove or disprove this claim.

Volatile components in cooked crabmeat have been studied by several investigators (Rayner et al., 1981; Hsieh et al., 1989; Flament, 1990; Matiella and Hsieh, 1990; Chung and Cadwallader, 1993). Hayashi et al. (1981) satisfactorily prepared a synthetic extract of boiled snow crab meat from 12 synthetic chemicals based on results from the triangle difference test (Jellinek, 1985).

Aroma extract dilution analysis (AEDA) is an olfactometric technique used widely in screening important aromas in an extract (Schieberle and Grosch, 1987; Gasser and Grosch, 1988; Blank et al., 1992). The aroma intensity unit is usually expressed as the flavor dilution (FD)-factor, which is defined as "the ratio of the concentration of a compound in the initial extract to that in the most diluted one in which the odor was detected by gas chromatography/olfactometry (GC/O)" (Blank and Grosch, 1991). The objective of the present study was to determine whether the flavor of lump and claw meats of blue crab differ as evaluated by sensory evaluation and AEDA.

## Materials and Methods

### Materials

Cooked blue crab claw and lump meats were purchased three separate times between August and September 1993 from a local seafood retail outlet in Baton Rouge, Louisiana. Meats were transported on ice to the Louisiana State University Department of Food Science and stored at 4°C until analyzed (less than 5 hr).

### Sensory evaluation of crab meats

In order to determine possible flavor differences between crab claw and lump meats, two sets of sensory

evaluations were carried out. Preliminary experiments suggested that warm crabmeat was preferred by the sensory panel over refrigerated or cold crabmeat when aroma quality was evaluated. Subsequent experiments were thus carried out in which warm (ca. 75°C) samples were presented to panelists. Furthermore, due to the distinct texture and color differences between the two crabmeats, panelists were able to distinguish samples even under red light or when blindfolded; therefore, experiments were carried out such that the effects of both qualities were eliminated or minimized. In this, aqueous crabmeat extracts were prepared to eliminate texture differences, and sample tubes were covered with aluminum foil to mask color differences. The sensory panel consisted of seven to 10 volunteers composed of faculty and students within the LSU Department of Food Science. All panelists had prior sensory-evaluation experience. Evaluations were conducted on crab claw and lump meats purchased from the same seafood outlet on the same day.

**Experiment 1.**

Each portion (ca. 5 g) of claw or lump meat was individually wrapped in aluminum foil, vacuum-packaged in a plastic bag (ca. 5 cm x 7 cm) and randomly numbered. All samples were cooled on ice during preparation and were evaluated within 24 hours. During each sensory evaluation session, four triangle difference tests, consisting of two

sets of duplicate samples, were performed by the panelists. Samples were warmed in a water bath (ca. 75°C) for at least 10 min before random distribution to the panelists. Sensory evaluation was performed in a temperature-regulated (23°C) room under red light to mask possible bias from package or sample color. Panelists were asked to massage each sample first with their fingers to simulate chewing before cutting open the package and evaluating its aroma.

**Experiment 2.**

Three hundred grams of each crabmeat plus 500 mL water was homogenized using a mixer (Cat. no. DIM24, General Electric, Bridgeport, CT) at low speed for 10 min in a stainless steel mixing bowl. Aqueous extract was obtained by filtering the homogenate through three layers of cheese cloth. Extract was further clarified by centrifugation at 3500 rpm (2000 x g) for 15 min using a RC5C Sorvall centrifuge (Ivan Sorvall, Inc., Norwalk, CT). Aqueous extract was pasteurized in a boiling (100°C) waterbath before transferring to culture tubes (7 mL/tube). Each tube was sealed with plastic wrap and the bottom half covered with aluminum foil to minimize bias from any sample color differences. Testing conditions were the same as previously described. Panelists were instructed to evaluate the tastes of the aqueous extracts by using one hand to block their noses. They were also

instructed to inhale and exhale using their mouths only. Water was provided to panelists for rinsing their mouths after each evaluation.

Additional experiments were carried out to determine if these aqueous extracts had any impact on the aroma perception of the panelists by instructing the panel to keep the sample in their mouths, hold it for 3 s, and exhale slowly into the headspace above the extract through their noses (Jellinek, 1985). Data from all sensory evaluations were analyzed statistically based on the table of significance in triangle tests of Roessler et al. (1978).

**Flavor extraction**

A sample of 200-g crabmeat was ground manually in a 2-L glass beaker using a pestle. Distilled water (200 mL) was added to facilitate extraction of water-soluble flavor components. Aqueous extract was recovered by pressing and filtering the mixture through three layers of cheese cloth into a 200-mL volumetric flask. The extract was further clarified by centrifuging at 3500 rpm (2000 x g) for 10 min and with 10 mL hexane to remove any oily pigment. Liquid supernatant was transferred into a 500-mL separatory funnel and extracted with 200 mL of 10% methanol in dichloromethane. The volume of the solvent extract was reduced to 5 mL under a gentle stream of

nitrogen, dried over 12-g anhydrous sodium sulfate, and further reduced to 0.4 mL.

**Gas chromatography/mass spectrometry and flame ionization detection/olfactometry (GC/MS-FID/O)**

The GC/MS system consisted of an HP 5790A GC equipped with a flame ionization detector (FID) and an HP 5970B mass selective detector (MSD)(Hewlett-Packard Co., Palo Alto, CA). Five μL of each extract was injected (splitless mode; 200°C injector temperature; 1 min valve delay) simultaneously into dual (closely matched) fused silica open tubular (FSOT) columns (Supelcowax 10, 60 m length x 0.25 mm i.d. x 0.25 μm film thickness; Supelco, Inc., Bellefonte, PA). Injector effluent, after passing through a FSOT precolumn (1 m length x 0.25 mm i.d. x 0.25 μm film thickness), was split 1:1 to each column using a glass Y-splitter. Column A was connected to the MSD, while the end of column B was split 1:1 to an FID and sniffing port supplied with humidified air. FID and sniffing port transfer line temperatures were 250 and 200°C, respectively. Helium was used as carrier gas at a linear velocity of 25 cm/s. It was necessary to partially restrict the flow between column A and MSD (using ca. 1 m length x 0.1 mm i.d. FSOT column) in order to achieve the same linear velocity for both columns. Oven temperature was programmed from 40 to 195°C at a rate of 2.0°C/min

with initial and final hold times of 5 and 40 min, respectively. MSD conditions were as follows: capillary direct MS interface temperature, 200°C; ion source temperature, 200°C; ionization voltage, 70 eV; mass range, 33-290 a.m.u.; scan rate, 1.67 scans/sec; and electron multiplier voltage, 1800 V.

**Aroma extract dilutions**

Serial dilutions were made from the 0.4 mL concentrated extracts in the ratio of 1:1 using dichloromethane as diluent. Each dilution was transferred to a new 2-mL amber vial equipped with a teflon-lined screw cap. Dilutions were stored at -80°C until analyzed.

**Gas chromatography/olfactometry (GC/O)**

The GC/O system consisted of an HP 5790A GC (Hewlett-Packard Co., Palo Alto, CA) equipped with a FSOT column (Supelcowax 10; 60 m x 0.32 mm i.d. x 0.25 $\mu$m film thickness; Supelco, Inc., Bellefonte, PA), an FID, and a sniffing port. Effluent from the end of the GC column was split 1:1 between the FID and the sniffing port. Each dilution (5 $\mu$L) was injected in the splitless mode (200°C injector temperature; 1 min valve delay). Oven temperature was programmed from 40°C to 195°C at a rate of 5°C/min with initial and final hold times of 5 and 40 min, respectively. Helium was used as the carrier gas at a

linear velocity of 25 cm/sec. Injector and detector temperatures were 200 and 250°C, respectively. GC/O was performed by two panelists familiar with crab flavor and the olfactometry technique. Each sample was replicated three times.

**Estimation of average FD-factor**

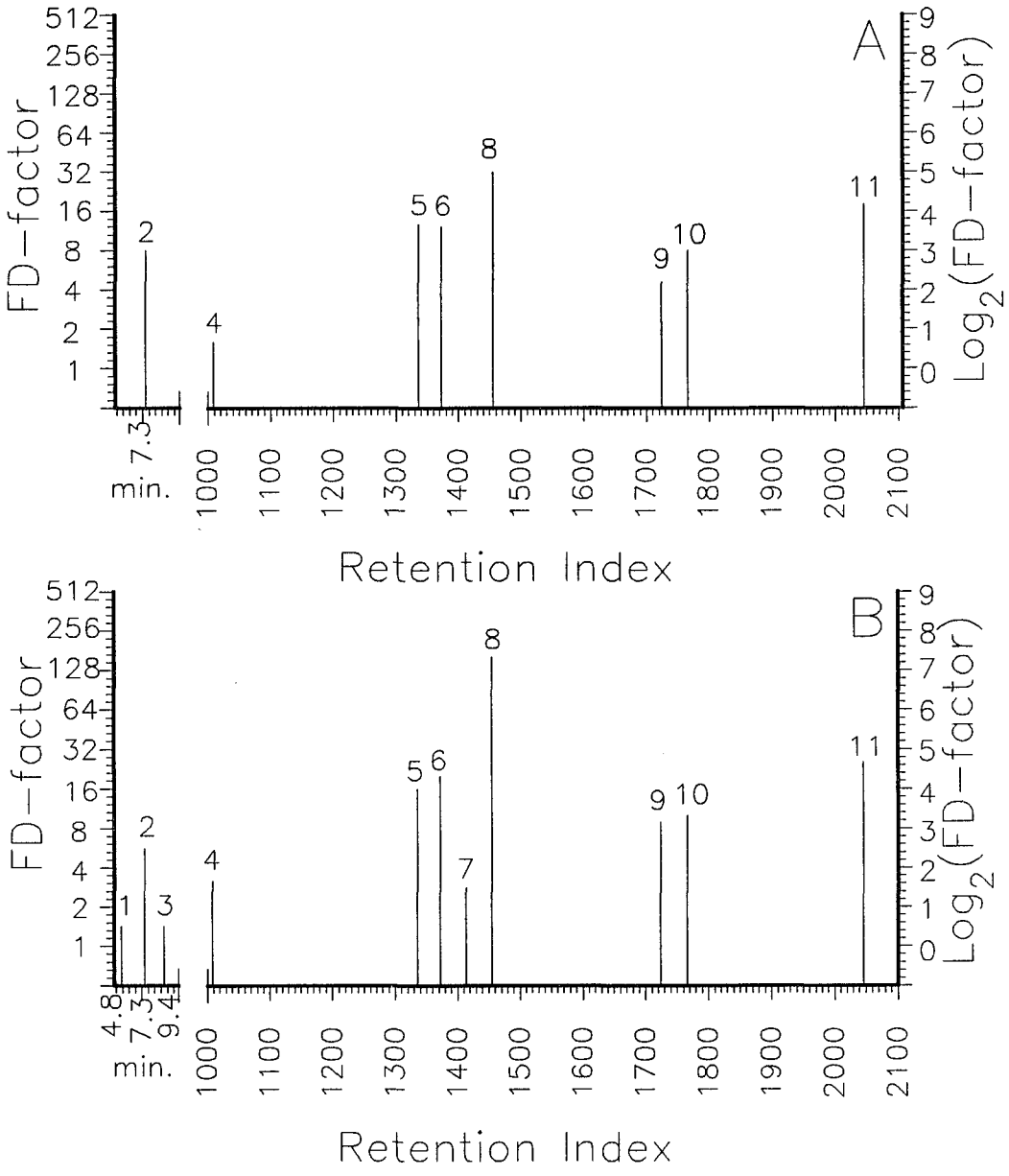
The average FD-factor of each flavor note was calculated by the arithmetic mean (N) of the $\log_2$(FD-factor) as described in chapter 4. (N) is readily converted back to FD-factor by:

(1) $$\text{FD-factor} = 2^N.$$

Figures 5.4A and B show FD-chromatograms of crab claw meat and lump meat, respectively. The left scale of the Y-axis is non-linear and represents the FD-factor. The right scale of the Y-axis is linear and expressed as $\text{Log}_2$(FD-factor). The retention index is expressed on the X-axis.

**Compound identification**

Identifications were based on comparison of GC retention indices (RI)(van den Dool and Kratz, 1963), mass spectra, and aroma properties of unknowns with those of authentic standard compounds analyzed under identical experimental conditions.




| | | | |
|---|---|---|---|
| 1 | fishy/ammonia | 7 | rice/popcorn |
| 2 | mushroom/solvent | 8 | meaty/salty/soy sauce |
| 3 | creamy/sour | 9 | pine/sweet/hay/dry seaweed |
| 4 | alkaline/rancid meal/yolk | 10 | nutty/popcorn |
| 5 | nutty/popcorn | 11 | sweet/fruity |
| 6 | metallic/rubber/gasoline | | |

**Figure 5.4 Flavor dilution (FD)-chromatograms of blue crab: (A) claw meat, and (B) lump meat.**

## Results and Discussion

### Aroma and taste of claw and lump crabmeats

Sensory evaluation results (Table 5.3) indicated no significant difference ($p<0.05$) between the aromas of claw and lump crabmeats in experiment 1. Although panelists were unable to distinguish differences between claw and lump crabmeat based on the aroma, when aqueous extracts of each sample were presented for tasting with their nose blocked with one hand, most panelists were able to tell the odd sample from each set, i.e., the tastes were significantly different ($p<0.05$). This suggests that aroma alone was not sufficient to allow panelists to differentiate between the two samples, but with the addition of taste sensation, the panelists could readily distinguish between them.

Farragut (1964) determined the proximate composition of blue crab in the Chesapeake Bay over a one-year period. During the months of August and September, the body meat had 15.6%, 81.65%, 1.05%, and 1.57% of protein, moisture, oil, and ash, respectively, on a wet-weight basis, while the claw meat had 15.5%, 82.4%, 1.0%, and 1.68%, respectively. The proximate compositions of the two types of meat were not dramatically different. However, at the molecular level, the differences between the lump and claw meats may be more obvious. George and Gopakumar (1987) compared the biochemical composition between claw and body

Table 5.3 Results of sensory evaluation (triangle difference test) of blue crab claw and lump meats

| Trial | Type | No. of Judgments | Correct | Incorrect | Level Tested[1] |
|---|---|---|---|---|---|
| Expt 1 | | | | | |
| 1 | aroma | 32 | 10 | 22 | 0.05 (-) |
| 2 | aroma | 36 | 16 | 20 | 0.05 (-) |
| Expt 2 | | | | | |
| 1 | taste | 28 | 15 | 13 | 0.05 (+) |
| 2 | taste | 40 | 30 | 10 | 0.001(+) |
| 1 | aroma | 28 | 15 | 13 | 0.05 (+) |
| 2 | aroma | 40 | 22 | 18 | 0.05 (+) |

[1] -: not significantly different, +: significantly different

meats of sand crab (*Scylla serrata*) and detected some major differences in the concentrations of glycogen, phosphorus, pentose, salt, protein, and amino acids. Body meat contained higher concentrations of glycogen, phosphorus, potassium, sarcoplasmic protein, and certain amino acids, e.g. glycine, alanine, aspartic acid, while claw meat contained more pentose, sodium, and amino acids such as valine, methionine, etc. Similar differences in composition would be expected for the two types of blue crab meats. In addition to the noticeable taste difference, both meats had distinct texture and color differences. Wasserman and Talley (1968) have implicated the influence of texture, color, and preparation of meat on the olfactory recognition processes; therefore, precautions were taken to eliminate or mask the texture and color differences during sensory evaluation.

Additional experiments on both extracts had surprising results relating to aroma perception. Panelists were able to distinguish differences based on aroma from the two extracts ($P<0.05$). Apparently, this contradicted the results in experiment 1. During breathing, inhaled air generally bypasses the regio olfactoria at the top inside of the nose where odor is sensed (Jellinek, 1985). If extracts of both meats are used, the odorant might be more readily released from the matrix in the mouth and create a more concentrated headspace of odorants. This, coupled

with the exhaling action, enabled the concentrated mass of odorant stream to pass the regio olfactoria for a more intense sensation. The massaging action in experiment 1 might not be very effective in releasing the odorant from the matrix. Even though the dilution effect of air on the sample was possible during sniffing, this was not likely the major cause since the panelists were asked to sniff the samples with their noses kept very close to the package after the samples were massaged and the packages cut open. It seemed more likely that the odorants were adsorbed strongly to the crab muscle matrix.

Results of the aroma evaluation were confirmed by the AEDA results. Both FD-chromatograms (Fig. 5.4A and 5.4B) showed a similar distribution but a different number of aroma-active notes. Eight and 11 characteristic aroma notes were detected in the claw and lump crabmeats, respectively. A salty/meaty/soy sauce-like note (No. 8) was the most intense aroma in both meats followed by a sweet/fruity note (No. 11), a metallic/rubber-/gasoline-like note (No. 6), and two nutty/popcorn-like notes (No. 5 and 10). The overall intensities of aroma notes were higher in lump crabmeat. The major difference in aroma sensation might be largely due to the three odorants in lump meat having fishy/ammonia-like (No. 1), creamy/sour (No. 3), and rice-/popcorn-like (No. 7) notes. These low-intensity notes might also be the cause of

insensitivity by panelists in experiment 1, but not in the latter part of experiment 2.

**Odorous compounds from aroma extract dilution analysis (AEDA)**

Whitfield et al. (1988) detected an iodoform-like off-flavor in some Australian crustacea and identified 2,6-dibromophenol as the causative agent. This compound also was found in marine algae and bryozoa on which the crustacea feed. Lately, Boyle et al. (1992) identified a group of bromophenols in saltwater Pacific salmon as well as in other seafoods. However, they reported that the sea-, brine-, iodine-like flavor notes were desirable and associated with saltwater fish and seafoods. They further reported detecting these compounds in high concentrations in blue crab. However, in the present experiment, detection of the above compounds did not occur.

The compound having the highest average $Log_2$(FD-factor) value was described as having a salty, meaty, soy sauce-like note (no. 8) in both types of crabmeat and was identified as 3-(methylthio)-propanal (Table 5.4). A Strecker aldehyde from methionine (Morton et al., 1960), this compound has a very low odor threshold of 0.2 ppb (Guadagni et al., 1972).

Table 5.4 Odorants detected in the extracts of blue crab claw and/or lump meats

| No.[1] | Aroma Description | RI/RT[2] | Average $Log_2$(FD-factor) Claw | Lump | Compound |
|---|---|---|---|---|---|
| 1 | fishy/ammonia | (4.8 min) | -[3] | 1.50 | trimethylamine |
| 2 | mushroom/solvent | (7.4 min) | 4.00 | 3.50 | unknown |
| 3 | creamy/sour | (9.4 min) | - | 1.50 | 2,3-butanedione |
| 4 | alkaline/rancid meal/yolk | 1008 | 1.67 | 2.67 | pyrrolidine |
| 5 | nutty/popcorn | 1336 | 4.67 | 5.00 | 2-acetyl-1-pyrroline |
| 6 | metallic/rubber/gasoline | 1372 | 4.60 | 5.33 | unknown |
| 7 | rice/popcorn | 1414 | - | 2.50 | unknown |
| 8 | meaty/salty/soy sauce | 1455 | 6.00 | 8.33 | 3-(methylthio)-propanal |
| 9 | pine/sweet/hay/dry seaweed | 1724 | 3.20 | 4.17 | unknown |
| 10 | nutty/popcorn | 1766 | 4.00 | 4.33 | unknown |
| 11 | sweet/fruity | 2046 | 5.17 | 5.67 | unknown |

[1] Numbers correspond to those in the flavor dilution (FD)-chromatograms.
[2] RI=retention index; RT=retention time.
Numbers in parentheses represent average retention times.
[3] - : odorant was not detected in the sample.

Another interesting note was described as having a sweet/fruity (no. 11) aroma and eluted very late during GC/O run. This note, along with notes no. 6 and 5, had similar FD-factors, while note no. 6 had a metallic/ rubber/gasoline-like aroma. Under the present chromatographic conditions, this note had an undesirable aroma. Nevertheless, its aroma might be diminished or masked by the other more desirable notes such as note nos. 6, 8, and 11. Metallic and fishy odorants were detected in oxidized butterfat by some researchers (Peers and Swoboda, 1977; Swoboda and Peers, 1977). Their investigations with a model system led them to conclude that both 1-octen-3-one and 1-octa-*cis*-5-dien-3-one were responsible for the metallic odor. In high concentrations, only 1-Octen-3-one possesses a metallic aroma; however, 1-octa-*cis*-5-dien-3-one produces this aroma at concentrations as low as 1 to $10^{12}$ dilutions. Since the present study involved cooked crabmeat, thermally generated compounds were expected to contribute to the crab aroma. Compound 2-acetyl-1-pyrroline (no. 5) was identified as imparting a nutty/popcorn-like note to the sample. Buttery et al. (1983) identified this compound in aromatic rice, and its odor threshold was reported to be 0.1 ppb.

It was uncertain why trimethylamine (TMA) was detected only in lump meat and its intensity was among the lowest in the sample. TMA was detected in claw meat in the

previous two experiments (Chapter 3 and 4), but not in this experiment. Such discrepancy might due to the volatility of the compound, and the extraction efficiency of the different extraction techniques employed. Josephson and Lindsay (1986) reported that TMA was an important contributor to the overall boiled crab-type aromas of fresh crab. Chung and Cadwallader (1993) compared the volatile compounds between blue claw meat and its CPB in which the average concentration of TMA was found to be 230 and 174 ng/g, respectively.

Pyrrolidine (no. 4) was the weakest aroma detected in both meats. This compound could have been thermally produced through Strecker degradation of proline or by bacteria (Allison and MacFarlane, 1989; Griffith and Hammond, 1989). Another thermally generated compound was 2,3-butanedione (no. 3), which was described as creamy and sour. Like TMA, this compound was identified only in the lump meat.

In conclusion, differences in the flavor of claw and lump crabmeat were confirmed, a finding which can be attributed to both volatile components and taste-active components. This conclusion was supported by the differences detected in the number of odorants as well as their relative intensity in the two crabmeat samples in the AEDA. Furthermore, it is possible that the distinct texture and color differences of the two types of

crabmeats might greatly influence the results of the overall organoleptic perception.

By comparing the present results with that of Chapter 4, three common odorants were found among these samples. They were identified as pyrrolidine, 2-acetyl-1-pyrroline, and 3-(methylthio)-propanal and could now be served as markers for the evaluation of CPB.

# CHAPTER 6

## EVALUATION OF BLUE CRAB (*Callinectes sapidus*) PROCESSING BY-PRODUCT AS A SOURCE FOR FLAVOR RECOVERY

### Introduction

Total product utilization is a trend in this century, and various ways have been investigated to optimize the use of seafood by-product. For example, researchers have reported the successful incorporation of recovered mince from underutilized crab claws into food products for institutional uses (Lee et al, 1993), as well as the use of the extracted carotenoid astaxanthin from crayfish processing by-product in fish feeds (Chen and Meyers, 1982). Jaswal (1990) investigated the potential use of hydrolyzed CPB using mineral acid. Recently, Cha et al. (1993) compared the volatile flavor components in snow crab cooker effluent to its effluent concentrate as well as in crayfish flavor concentrate prepared at 85°C to that at 100°C (Cha et al., 1992).

The objective of the present study was to explore the use of blue crab processing by-product (CPB) as a potential feedstock for flavor recovery, and to optimize the recovery by monitoring the quantities of selected odorants at different conditions.

## Materials and Methods

### Materials

Blue crab processing by-product (CPB) was obtained from a seafood processor in St. Martinville, Louisiana, in October 1993. Two kg of CPB was randomly picked from a larger stock and manually ground in a stainless steel bowl with a plastic pestle. Samples were vacuum packaged (200g) and stored frozen (-20°C) until extraction.

### Bone extract (BX)

Two hundred grams of frozen CPB was thawed under running tap water for 30 min and ground. CPB was rinsed with a total of 200 mL (10 x 20 mL) distilled water to remove odorants and their precursors that might attach to the CPB matrix. Hard tissue and filtrate (rinse water) were collected separately by filtering the CPB through three layers of cheese cloth. Hard tissue was transferred to a 1000-mL round-bottom flask and 300 mL of distilled water was added for distillation. The distillation unit consisted of a condenser (0°C), a distillation head, a bent adapter, a thermometer, and a heating mantle connected to a rheostat (75%). Two hundred mL distillate was collected directly in a 500-mL separatory funnel and was subsequently extracted with 200 mL (10 x 20 mL) dichloromethane containing 10% (V/V) methanol. Solvent extract prepared from the hard tissue (BX) was

concentrated under a stream of nitrogen to 0.4 mL for preparation of aroma extract dilutions. Triplicate extractions were prepared.

**Rinse water extract (RWX)**

Rinse water (200 mL) from hard tissue was centrifuged with 10 mL hexane at 2000 x g and 4°C for 15 min to remove any lipids present. Aqueous supernatant (RWX) was recovered, and the hexane fraction was discarded. Extraction of rinse water was done as for BX.

**Aroma extract dilutions**

The 0.4-mL concentrate of each extract was diluted with dichlormethane in a 1-to-1 ratio. Dilutions were stored at -80°C until analyzed.

**Gas chromatography/olfactometry (GC/O)**

An HP 5790A GC (Hewlett-Packard Co., Palo Alto, CA) equipped with a capillary column (Supelcowax 10; 60 m x 0.32 mm i.d.; 0.25 $\mu$m film), flame ionization detector (FID), and olfactometer (O) were used. GC temperature program was as follows: 40°C for 5 min, 195°C for 40 min, and ramp rate at 5°C/min. Carrier gas (He) flow rate was 25 cm/sec and was split at a ratio of 1:1 into the FID and O. Five $\mu$L samples were analyzed. Injector and detector temperatures were 200°C and 250° C, respectively.

Splitless valve time was maintained for 60 sec. Triplicate samples were prepared and evaluated by a panelist familiar with the olfactometry technique and the sample.

**Gas chromatography/mass spectrometry (GC/MS)**

An HP 5790A GC (Hewlett-Packard Co., Palo Alto, CA) equipped with a capillary column (Supelcowax 10; 60 m x 0.25 mm i.d.; 0.25 $\mu$m film) was coupled with an HP 5970B mass selective detector. Gas chromatograph conditions were as follows: helium (carrier gas, 99.9% purity) flow rate: 25 cm/sec; oven temperature program: 40°C (5 min) to 195°C at 2°C/min., hold for 40 min; injector temperature: 200°C. HP 5970 MSD parameters were: mass range: 33-290 a.m.u.; ionization voltage: 70 eV; interface temperature, 200°C; scan rate: 1.67 scans/sec. A 5-uL sample was injected in the splitless mode.

**Estimation of average FD-factor**

The estimation of average FD-factor for each flavor note had been described in the previous chapter.

**Compound identification**

Aroma-compound identifications were based on comparison of GC-retention indices (RI) (van den Dool and Kratz, 1963), mass spectra, and odor properties of

unknowns with those of authentic standard compounds under identical experimental conditions.

**Distillation and reflux**

In order to determine whether there were any differences in the quantitative recovery of selective odorants under different heating conditions, different heating processes, and different sample types, two additional experiments were carried out. Figure 6.5 shows the combined scheme of sample preparation for both experiments.

**Experiment 1.--Distillation**

Two hundred grams of CPB were homogenized in a Waring blender (model 91-263, Dynamics Corp. of America, New Hartford, CT) with 200 mL distilled water. The sample was then filtered through three layers of cheesecloth to remove the hard tissue, and the filtrate was centrifuged with 10 mL hexane at 2000 x g and 4°C for 15 min to divide the material into the liquid supernatant (SP) and the solid residue (RE) portions. Both SP and RE were divided equally into subportions as control and experimental groups.

One hundred and 200 mL distilled water were added to the control groups of both SP and RE, respectively, so that a final volume of 200 mL was reached. Both controls were extracted with 50 mL of 10% methanol in redistilled

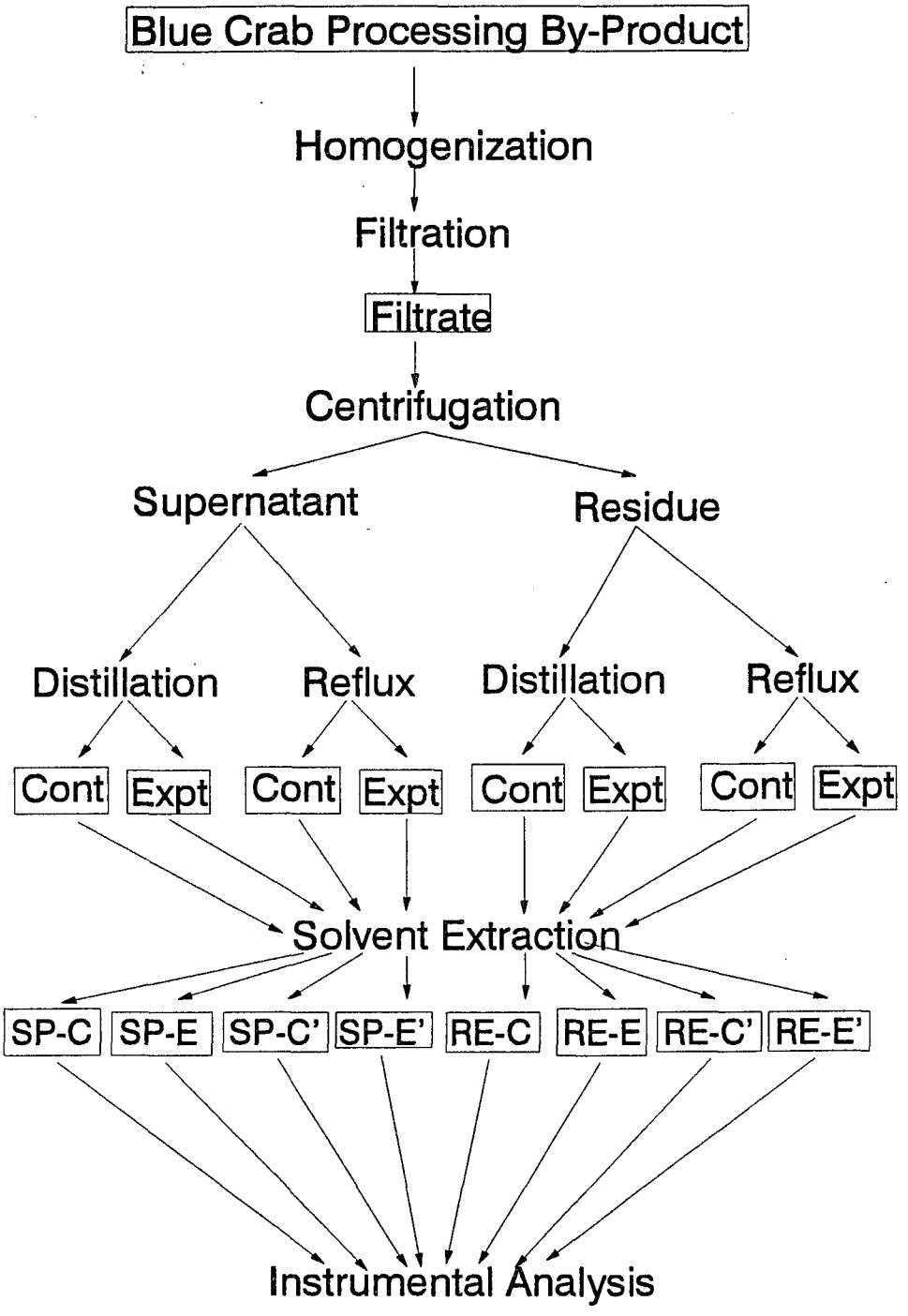



Figure 6.5 Flow diagram of crab by-product sample preparation.

dichloromethane after the addition of 4.585 μg 2,4,6-trimethylpyridine (TMP) (internal standard). The resulting extracts from SP and RE were labelled as SP-C and RE-C, respectively.

Similarly, volumes of 200 and 300 mL distilled water were added to the experimental groups of SP and RE, respectively. The additional amount of distilled water was added in the experimental groups to prevent dehydration of the sample during distillation. The experimental groups were subjected to distillation and extraction as described previously for BX. Subsequent extracts were marked as SP-E and RE-E.

All extracts were dried over anhydrous sodium sulfate (12 g) and concentrated to a final volume of 0.1 mL under a gentle stream of purified nitrogen. Extracts were stored in a freezer (-20°C) until analyzed. The experiment was replicated four times.

**Experiment 2.--Reflux**

Similar procedures were used as for the distillation, except the glassware was configured for continuous reflux. Two condensers were mounted vertically side by side with their vents covered with 2-cm-diameter watch glasses. Samples were divided equally and the same amount of water was used in the controls as in the distillation experiment described above. For the experimental groups, 100 and 200 mL distilled water were introduced to the SP and RE,

respectively. Each reflux was carried out for one hour. Solvent extractions were done as described previously. Extracts prepared from the two controls and from the two experimental groups were labelled as SP-C', RE-C', SP-E'and RE-E', respectively. Four replicate samples were prepared.

**Selected ion monitoring (SIM)**

Five compounds (2,3-butanedione [BD], pyrrolidine [PYR], (Z)-4-heptenal [HPT], 2-acetyl-1-pyrroline [2-AP], and 3-(methylthio)-propanal [3-MP]) were chosen for quantification based on their consistent detections in the BX during the GC/O experiment. Besides, three compounds including PYR, 2-AP and 3-MP, have been determined to be important markers for cooked crabmeat aroma from the results of the previous two experiments in Chapter 4 and 5. The internal standard (TMP) was also monitored. The following ions, mass/charge (m/z), were monitored for each compound: (BD)-43, 86; (PYR)-43, 70, 71; (HPT)- 41, 68, 84, 112; (2-AP)-43, 83, 111; (2-MP)-48, 61, 76, 104; and TMP-70, 106, 121. GC/MS conditions were the same as before except that the oven-temperature program was modified as follows: 40°C (5 min) to 195°C at 5°C/min., then hold for 60 min. In order to facilitate statistical analysis, relative peak area ratios between the selected compounds and the internal standard (IS) were calculated by dividing

the area sum of all selected ions for each compound by that of IS.

**Statistical analysis**

Data was analyzed statistically using a randomized block design (RBD) with a factorial arrangement of treatments. Statistical analysis of data was conducted using analysis of variance (ANOVA) with the general linear models (GLM) procedure (SAS Institute, Inc., 1989). Least-square means (LS Means) of the relative-area ratios between each compound and the internal standard for each experimental condition were calculated, and significant differences were evaluated by the t-statistic method.

## Results and Discussion

Odorants detected by GC/O in BX and RWX are summarized in Table 6.5. These results showed that five and three compounds found in BX and RWX, respectively, could be found in crabmeat. Judging from the presence of all markers in BX and RWX and the number of other odorants detected, CPB is confirmed to be a potent source for flavor recovery.

Table 6.5 Odorants detected in the extracts of blue crab processing by-product and/or rinse water

| No. | RI/RT[1] | Aroma Description | Compound | Average $Log_2$(FD-factor)[2] | |
|---|---|---|---|---|---|
| | | | | Bone (BX) | Rinse Water (RWX) |
| 1 | (8.63 min) | diacetyl | 2,3-butanedione | 5.55 | -[3] |
| 2 | 1003 | alkaline | pyrrolidine | 3.33 | 4.67 |
| 3 | 1052 | diacetyl | 2,3-pentanedione | 1.33 | - |
| 4 | 1079 | herbal/woody/coffee | | 5.33 | - |
| 5 | 1237 | potato | (Z)-4-heptenal | 6.67 | - |
| 6 | 1263 | toffee candy | | 0.67 | - |
| 7 | 1288 | woody/herbal/mushroom | | - | 2.00 |
| 8 | 1297 | mushroom | | 3.33 | - |
| 9 | 1334 | nut/sweet/popcorn | 2-acetyl-1-pyrroline | 7.33 | 7.33 |
| 10 | 1351 | dry cloth | | 0.67 | - |
| 11 | 1359 | bad onion | | 0.67 | - |
| 12 | 1371 | metal | | 6.67 | 7.33 |
| 13 | 1420 | nut/raw peanut | | 2.00 | 2.67 |
| 14 | 1427 | raw peanut/peanut skin | | 2.67 | 0.00 |
| 15 | 1456 | soy sauce | 3-(methylthio)-propanal | 11.33 | 4.67 |
| 16 | 1506 | dry wood | | 1.33 | - |
| 17 | 1573 | jasmine tea | | 4.00 | - |
| 18 | 1587 | floral/sweet | | 3.33 | - |
| 19 | 1604 | something | | 5.33 | - |
| 20 | 1630 | burnt hair | | 5.33 | - |
| 21 | 1651 | ammonia | | 6.00 | - |
| 22 | 1728 | limonine/fruity/minty | | 5.33 | - |
| 23 | 1737 | hay | | - | 2.00 |
| 24 | 1769 | nut/sweet | | 4.00 | - |
| 25 | 1926 | hay/dry seaweed | | 3.33 | - |
| 26 | 1939 | hay/dry seaweed | | 1.33 | - |

[1] RI=retention index; RT=retention time. Numbers in parentheses represent average retention times.
[2] FD-factor: flavor dilution factor
[3] - : odorant was not detected in the sample.

**Bone extract (BX) and rinse-water extract (RWX)**

Both BX and RWX came from the same portion of CPB. Many odorants (26) were detected in BX. Further, more odorants were detected in BX (26) than in RWX (8). Since the rinse water originated from CPB, it is easy to explain the similarity in the odorants detected in the two extracts. These results suggest that substantial amounts of components retained by the rinsed CPB reacted or were released during distillation of the rinsed CPB. These components could exist as precursors that formed odorants during distillation or as odorants physically bound with the matrix that were released during distillation. Lee et al. (1993) reported that 58.41% blue crab composite mince (with gills) was recovered from CPB using mechanical separation. The major portion of this mince was protein (15.32%). Loss of water-soluble proteins by migration or exposure to water during processing was thought to cause a decrease in blue crab flavor (Dowdie and Biede, 1983). However, it is possible that some other important hydrophilic components also might be lost when exposed to water.

RWX had an aroma impression dominated by undesirable dry hay-like, bitter, and green notes. AEDA revealed eight odorants dominating RWX aroma with a metallic note (No.12) having the highest $Log_2$(FD-factor) value of seven (Table 6.5). This might be the major cause of the undesirable

aroma impression in RWX. Such a characteristic metallic note was also detected in the previous experiment with claw and lump crab meats. In Chapter 5, the metallic note was masked by other more desirable notes described as popcorn-like and soy sauce-like. In fact, these notes were also detected in both BX and RWX.

Desirable odorants, such as nutty, sweet, popcorn-like aroma (No. 9) and soy sauce-like aroma (No. 15), were detected in RWX as mentioned above. Both had a very desirable aroma quality and were identified as 2-acetyl-1-pyrroline (2-AP) and 3-(methylthio)-propanal (3-MP), respectively. Further, pyrrolidine (PYR) was identified in both BX and RWX and described as having an alkaline-like note. These three compounds were considered to be important markers for cooked blue crab aroma.

Both 2-AP and 3-MP have been reported in aromatic rice (Buttery et. al, 1983) and in shoyu flavor concentrate (Nunomura et al., 1976), respectively. Methionine has been shown to be an important precursor of 3-MP, which forms as a Strecker aldehyde by reaction with dicarbonyls produced from the Maillard reaction (Morton et al., 1960). Guadagni et al. (1972) reported the odor threshold of 3-MP to be 0.2 ppb in both water and oil. This discovery allows one to speculate on the composition of the components, such as the presence of sulfur-containing compounds.

Since the initial part of the experiment confirmed the presence of some important odorants in CPB, a second set of experiments was designed to monitor the recovery of several selected odorants at different experimental conditions.

**Comparison of selected odorants at different conditions**

Results from the statistical analysis are shown in Table 6.6. Among the five compounds monitored, the least-square means of HPT and BD were significantly higher in the residue, while 2-AP and 3-MP were higher in the supernatant. There was no significant difference of PYR in either the supernatant or residue. Since the recovery of two out of three markers was found statistically higher in the supernatant, it seemed that using supernatant as the starting material for flavor recovery was a better option.

Distillation was more effective in the overall recovery of 3-MP and HPT than reflux. There was no significant difference in the two methods for the other compounds. Distillation might be superior to reflux in that a clear distillate, not contaminated by the original matrix, was recovered. Furthermore, recovery of most of the higher boiling, potentially undesirable aromas, could be minimized.

Heating was necessary to recover more aroma compounds during both distillation and reflux, as shown in the

Table 6.6 Statistical results of selected compounds at different conditions

| | Least-Square Means of Selected Compounds | | | | |
|---|---|---|---|---|---|
| Condition | BD[1] | PYR[2] | HPT[3] | 2-AP[4] | 3-MP[5] |
| Supernatant | 3.2142 | 0.0019 | 0.035 | 0.0064*** | 0.080* |
| Residue | 6.3817* | 0.0027 | 0.060** | 0.0008 | 0.014 |
| Distillation | 4.2920 | 0.0020 | 0.075*** | 0.0041 | 0.093** |
| Reflux | 5.3039 | 0.0026 | 0.020 | 0.0031 | 0.001 |
| Control | 4.1914 | 0.0020 | 0.023 | 0.0007 | 0.002 |
| Experimental | 5.4045 | 0.0026 | 0.073*** | 0.0064*** | 0.092** |

[1] BD: 2,3-butanedione
[2] PYR: pyrrolidine
[3] HPT: (Z)-4-heptenal
[4] 2-AP: 2-acetylpyrroline
[5] 3-MP: 3-(methylthio)-propanal
* significantly different (P<0.05)
** significantly different (P<0.01)
*** significantly different (p<0.001)

comparison between the control and experimental groups. Three out of five selected compounds were found to have significantly higher recovery after heating. Least-square means of experimental groups were generally higher than the corresponding control groups, indicating that heating was in fact very critical in releasing more odorants.

In conclusion, CPB was found to be an important source of raw material for potential crab-flavor recovery. Even though it was observed that some selected compounds increased in recovery during distillation and reflux, we were unable to conclude whether they were released from the CPB matrix or produced from precursors. Heating certainly increased the recovery of most odorous compounds. However, the effectiveness in producing selected compounds was not the same for both supernatant and residue. Nevertheless, with markers as references, supernatant was a better starting material for flavor recovery than residue. Finally, distillation was a better heating process than reflux for recovering more selected odorous compounds.

# CHAPTER 7

## SIGNIFICANT FINDINGS AND CONCLUDING REMARKS

Though speculation has existed relating to the utilization of crab processing by-product (CPB) as a potential starting material for crab-flavor recovery, a thorough literature review revealed that relevant information is scarce. The taste-active components of snow crab have been thoroughly investigated (Hayashi et al., 1978; Konosu et al., 1978; Hayashi et al., 1979; Hayashi et al., 1981; Konosu and Yamaguchi, 1982). These studies concluded that 12 out of the initial 44 detectable taste-active components in snow crab were indispensable in the replication of a similar flavor using pure chemicals. Recently, Hayashi et al. (1990) concentrated on the relationship between taste-active components and aroma in snow crabs, suggesting minor components might act as precursors for the aroma compounds. Limited information is available with regards to the volatile flavor of blue crab (*Callinectes sapidus*) (Hsieh et al., 1989; Matiella and Hsieh, 1990), and none for the CPB. This report is the first of its kind in characterizing the flavor of blue crab and its CPB with emphasis in the areas of instrumental analysis, olfactometry, and sensory

evaluation, from which the usefulness of CPB as a raw material for volatile flavor recovery could be revealed.

Significant findings of this research include the following: (1) establishment of the importance of CPB as a source for flavor recovery based on the results of instrumental analyses (Chapter 3); (2) defining crab claw meat aroma using gas chromatography/olfactometry (GC/O) (Chapter 4); (3) confirming the qualitative and quantitative differences between different types of crabmeat (claw and lump) by sensory evaluation and GC/O (Chapter 5); and (4) confirming CPB as a good source for crab flavor recovery (Chapter 6).

The long-term goal of the present study was to demonstrate that CPB can be used to prepare flavor-related materials. However, without prior knowledge of the quality of CPB, it would be risky to conclude CPB per se was a good starting material for further processing. This was the main reason behind the first experiment (Chapter 3). Results obtained from the extractions of claw meat and CPB were very encouraging. Almost all compounds that were detected in both samples were quantitatively higher in CPB. However, in one problem that arose, it could not be determined whether CPB was suitable for flavor recovery based primarily on the analytical results. There was always the possibility that some important flavor

components with low thresholds and low concentrations were not detected, or were overlooked, during the identification procedure. Another difficulty emerged when it was realized that there were no markers available from the literature that could be used to evaluate CPB. With this in mind, it was necessary to obtain a basic understanding of crab flavor so that appropriate markers could be used for CPB quality assessment. The second experiment (Chapter 4) achieved this objective by carrying out the GC/O technique known as aroma extract dilution analysis (AEDA). Though the analysis was time consuming, seven odorants were detected that better defined the aroma of blue crab claw meat. Subsequent experiments (Chapter 5) were conducted to clarify whether different types of crabmeat (claw and lump) have different sensory properties. Results from both sensory evaluation and AEDA indicated there were differences between them, although the qualitative differences were slight. The subtle flavor difference of lump meat might be due to three additional odorants detected. By comparing the odorants detected in the last two experiments (Chapter 4 and 5), at least three odorants were commonly found in both experiments, including pyrrolidine, 2-acetyl-1-pyrroline, and 3-(methylthio)-propanal. These compounds are believed to be indispensable to crabmeat aroma. With crabmeat aroma defined, the usefulness of CPB could then be evaluated

(Chapter 6). Additional experiments were done using selected ion monitoring (SIM) to monitor those markers under different heating conditions (control vs experiment); under different heating processes (distillation vs reflux); and by evaluating different starting materials (supernatant vs residue) of CPB. It was concluded that CPB was a good raw material for crab-flavor recovery, and heating with distillation achieved the best results.

This research characterized the flavor of both crabmeat and CPB and confirmed the usefulness of the latter as a potential flavor-recovery source. However, there are other implications from these findings. For example, markers can be used to monitor the quality of crabmeat products such as canned foods. Also, they can serve as quality-control indicators during the recovery of flavor from CPB, as well as during formulation of new food products requiring crab-like flavor.

Although no attempts were made in this research to prepare an actual crab-flavor extract from CPB or to consider the economics of such practices, it is hoped that this research will stimulate the interest of present and future researchers to investigate use of CPB for flavor production. In addition, the author hopes this will facilitate future research in this area to achieve the objective of total resource utilization.

## BIBLIOGRAPHY


Abbott, N., Etievant, P., Issanchou, S., Langlois, D. 1993. Critical evaluation of two commonly used techniques for the treatment of data from extract dilution sniffing analysis. J. Agric. Food Chem. 41: 1698-1703.

Acree, T.E. 1993. Gas chromatography-olfactometry. In *Flavor Measurement*, C.T. Ho and C.H. Manley (Ed.), p. 77-94. Marcel Dekker, Inc., New York, NY.

Acree, T.E., Barnard, J., and Cummingham, D. 1984. A procedure for the sensory analysis of gas chromatographic effluent. Food Chem. 14: 273-286.

Allison, C. and MacFarlane, G.T. 1989. Influence of pH, nutrient availability, and growth rate on amine production by *Bacteroides fragilis* and *Clostridium perfringens*. Appl. Environ. Microbiol. 55: 2894-2898.

Ames, J. and Macleod, G. 1984. Volatile components of an unflavored textured soy protein. J. Food Sci. 49: 1552-1557.

Ando, M. and Osawa, N. 1988. Processed seafoods (e.g., crab meat) and flavourings used. New Food Industry 30: 9-15.

ASTM. 1968. *Manual on sensory testing methods*, ASTM special technical publication No. 434. American Society for Testing and Materials, Philadelphia, Pa.

Babbitt, J.K. 1992. Crabs and crab processing. In *Encyclopedia of Food Science and Technology*, Y.H. Hui (Ed.), p. 513-517. John Wiley & Sons, Inc., New York, NY.

Blank, I. and Grosch, W. 1991. Evaluation of potent odorants in dill seed and dill herb (*Anethum Graveolens L.*) by aroma extract dilution analysis. J. Food Sci. 56: 63-67.

Blank, I., Sen, A. and Grosch, W. 1992. Potent odorants of the roasted powder and brew of Arabica coffee. Z. Lebensm. Unters. Forsch. 195: 239-245.

Bowman, T.E. 1986. Crab. In *The Encyclopedia Americana*--International Edition, p. 139-141. Grolier Inc., Danbury, Conn.

Boyle, J.L., Lindsay, R.C. and Stuiber, D.A. 1992. Bromophenol distribution in salmon and selected seafoods of fresh- and saltwater origin. J. Food Sci. 57: 918-922.

Buttery, R.G., Ling, L.C., Juliano, B.O. and Turnbaugh, J.G. 1983. Cooked rice aroma and 2-acetyl-1-pyrroline. J. Agric. Food Chem. 31: 823-826.

Cadwallader, K.R., Baek, H.H., Chung H.Y. and Moody, M.W. 1994. Contribution of lipid-derived components to the flavor of alligator meat. In *Lipids and Food Flavors*, C.T. Ho and T.G. Hartman (Ed.), ACS Symposium Series. (In Press).

Cain, W.S. 1977. Differential sensitivity for smell: "Noise" at the nose. Science 195: 796-798.

Cerny, C. and Grosch, W. 1992. Evaluation of potent odorants in roasted beef by aroma extract dilution analysis. Z. Lebensm. Unters. Forsch. 194: 322-325.

Cha, Y.-J., Baek, H.-H. and Hsieh,T. C.-Y. 1992. Volatile components in flavour concentrates from crayfish processing waste. J. Sci. Food Agric. 58: 239-248.

Cha, Y.J., Cadwallader, K.R. and Baek, H.H. 1993. Volatile flavor components in snow crab cooker effluent and effluent concentrate. J. Food Sci. 58: 525-530.

Chang, S.S. and Peterson, R.J. 1977. Recent developments in the flavor of meat. J. Food Sci. 42: 298-305.

Chen, H.M. and Meyers, S.P. 1982. Extraction of astasxanthin pigment from crawfish waste using a soy oil process. J. Food Sci. 47: 892-896, 900.

Chung, H.Y. and Cadwallader, K.C. 1993. Volatile components in blue crab (*Callinectes sapidus*) meat and processing by-product. J. Food Sci. 58: 1203-1207, 1211.

Devos, M., Patte, F., Rouault, J., Laffort, P. and Van Gemert, L.J. (Ed.). 1990. *Standardized Human Olfactory Thresholds*. IRL press, NY.

Dowdie, O.G. and Biede, S.L. 1983. Influence of processing temperature on the distribution of tissue and water-soluble proteins in blue crabs (*Callinectes sapidus*). J. Food Sci. 48: 804-807, 812.

Dravnieks, A. (Ed.). 1985. *Atlas of Odor Character Profiles*. ASTM Data Series DS 61. American Society for Testing and Materials, Philadelphia, PA.

Evers, W.J., Heinsohn, H.H.,Jr., Mayers, B.J. and Sanderson, A. 1976. Furans substituted at the three position with sulfur. Ch. 11. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors*, ACS Symposium Series 26, G. Charalambous and I. Katz (Ed.). p. 184-193. American Chemical Society, Washington, DC.

Farragut, R.N. 1964. Proximate composition of Chesapeake Bay blue crab (*Callinetes sapidus*). J. Food Sci. 30: 538-544.

Flament, I. 1990. Analysis of boiled crab meat flavour: identification of new alcoholic constituents. Lebensm.-Wiss. u.-Technol. 23: 274-275.

Fors, S. 1983. Sensory properties of volatile Maillard reaction products and related compounds. Ch. 12. In *Maillard Reaction in Food and Nutrition*, ACS symposium Series 215, G.R. Waller and M.S. Feather (Ed.), p. 185-286. American Chemical Society, Washington, DC.

Gasser, U. and Grosch, W. 1988. Identification of volatile flavour compounds with high aroma values from cooked beef. Z. Lebensm. Unters. Forsch. 186: 489-494.

Gates, K.W. and Parker, A.H. 1992. Characterization of minced meat extracted from blue crab picking plant by-products. J. Food Sci. 57: 267-270,292.

Genetelli, E.J. and Cirello, J. (Ed.). 1976. *Gas and Leachates from Landfills, Formation, Collection and Treatment*. U.S. Environmental Protection Agency, Cincinnati, OH.

George, C. and Gopakumar, K. 1987. Biochemical studies on crab *Scylla Serrata*. Fishery Technol. 24: 57-61.

Griffith, R. and Hammond, E.G. 1989. Generation of Swiss cheese flavor components by the reaction of amino acids with carbonyl compounds. J. Dairy Sci. 72: 664-613.

Grosch, W. 1982. Lipid degradation products and flavour. Ch. V. Part A. Introduction. In *Developments in Food Science 3A*, I.D. Morton, and A.J. MacLeod (Ed.), p. 325-398. Elsevier Sci. Publishing Co., Inc., New York, NY.

Guadagni, D., Buttery, R. and Turnbaugh, J. 1972. Odor thresholds and similarity ratings of some potato chip components. J. Sci. Food Agric. 23: 1435-1444.

Güntert, M., Brüning, J., Emberger, R., Hopp, R., Köpsel, M., Surgurg, H., and Werkhoff, P. 1992. Thermally degraded

thiamin: a potent source of interesting flavor compounds. Ch. 11. In *Flavor Precursor: Thermal and Enzymatic Conversions*, ACS Symposium Series 490, R. Teranishi, G.R. Takeoka, and M. Güntert (Ed.), p. 140-163. American Chemical Society, Washington, DC.

Guth, H. and Grosch, W. 1991. A comparative study of the potent odorants of different virgin olive oils. Fat Sci. Technol. 9: 335-339.

Guth, H. and Grosch, W. 1992. Detection of furanoid fatty acids in soya-bean oil--cause for the light-induced off-flavour. Fat Sci. Technol. 7: 249-255.

Ha, J.K. and Lindsay, R.C. 1991. Volatile alkylphenols and thiophenol in species-related characterizing flavors of red meats. J. Food Sci. 56: 1197-1202.

Hanover, L.M., Webb, N.B., Jowell, A.J., and Thomas, F.B. 1973. Effects of cooking and rinsing on the protein losses from blue crabs. J. Milk Food Technol. 36: 409-413.

Hanover, L.M., Webb, N.B., Howell, A.J., and Thomas, F.B. 1975. BOD, COD, and TOC values for liquid wastes from selected blue crab pilot processes. J. Milk Food Technol. 38: 155-158.

Hayashi, T., Asakawa, A., Yamaguchi, K., and Konosu, S. 1979. Studies on flavor components in boiled crabs. III. Sugars, organic acids, and minerals in the extracts. Bull. Japan. Soc. Sci. Fish. 45: 1325-1329.

Hayashi, T. Ishii, H., and Shinohara, A. 1990. Novel model experiment for cooking flavor research on crab leg meat. Food Rev. Int. 6: 521-536.

Hayashi, T., Yamaguchi, K., and Konosu, S. 1978. Study on the flavor components in boiled crabs--II. Nucleotides and organic bases in extracts. Bull. Jap. Soc. Sci. Fish. 44: 1357-1362.

Hayashi, T., Yamaguchi, K., and Konosu, S. 1981. Sensory analysis of taste-active components in the extract of boiled snow crab meat. J. Food Sci. 46: 479-483.

Hites, R.A. and Biemann, K. 1970. Computer evaluation of continuously scanned mass spectra of gas chromatographic effluent. Anal. Chem. 42: 855-860.

Hodge, J.E. 1967. Origin of flavour in foods. Nonenzymic browning reactions. Ch. 22. In *Chemistry and Physiology of*

*Flavours*, H.W. Schultz, E.A. Day, L.M. Libbey (Ed.), p.465-491. AVI Publishing Co., Westport, CT.

Hornstein, I. and Crowe, P.F. 1960. Flavor studies on beef and pork. J. Agric. Food Chem. 8: 494-498.

Hsieh, T.C.-Y., Vejaphan, W., Williams, S.S., and Matiella, J.E. 1989. Volatile flavor components in thermally processed Louisiana red swamp crayfish and blue crab. Ch. 36. In *Thermal Generation of Aromas*, ACS symposium series 409, Parliment, T.H., McGorrin, R.J. and Ho, C.T. (Ed.). p. 386-395. American Chemical Society, Washington, DC.

Hsu, C.-M. Peterson, R.J., Jin, Q.Z., Ho, C.-T. and Chang, S.S. 1982. Characterization of new volatile compounds in the neutral fraction of roasted beef flavor. J. Food Sci. 47: 2068-2069, 2071.

Hwang, S.S., Carlin, J.T., Bao, Y., Hartman, G.J. and Ho, C.T. 1986. Characterization of volatile compounds generated from the reactions of aldehydes with ammonium sulfide. J. Agric. Food Chem. 34: 538-542.

Ingham, S.C. and Moody, M.W. 1990. Enumeration of aerobic plate count and *E. Coli* during blue crab processing by standard methods, Petrifilm™, and Redigel™. J. Food Prot. 53: 423-425.

Jaswal, A.S. 1990. Amino acid hydrolysate from crab processing waste. J. Food Sci. 55: 379-380, 397.

Jaworski, E. (Ed.). 1972. *The Blue Crab Fishery, Barataria Estuary*. Louisiana Center for Wetland Resources. Louisiana State University, Baton Rouge, La.

Jellinek, G. (Ed.). 1985. *Sensory Evaluation of Food: Theory and Practice*. VCH Publishers, Deerfield Beach, FL.

Josephson, D.B. and Lindsay, R.C. 1986. Enzymic generation of volatile aroma compounds from fresh fish. Ch. 17. In *Biogeneration of Aroma*, ACS Symposium Series 409, T.H. Parliment, R.J. McGorrin, and C.T. Ho.(Ed.), p. 386-395. American Chemical Society, Washington, DC.

Jung, J.-P., Sen, A. and Grosch, W. 1992. Evaluation of potent odorants in parsley leaves [*Petroselinum crispum* (Mill.) Nym. ssp. crispum] by aroma extract dilution analysis. Lebensm.-Wiss. u.-Technol. 25: 55-60.

Kallio, H., Tuomola, M., Pessala, R., and Vilkki, J. 1990. Headspace GC-analysis of volatile sulfur and carbonyl compounds in chive and onion. Ch. 1. In *Flavour Science and Technology*, Y. Bessiere and A.F. Thomas (Ed.), p. 57-60. John Wiley & Sons Ltd., New York, NY.

Keithly Jr., W.R., Roberts, K.J. and Liebzeit, A.W. 1988. *Louisiana Blue Crab--Production, Processing, and Markets*. Louisiana Sea Grant College Program, Center for Wetland Resources, Louisiana State University, Baton Rouge, LA.

Kim, H.R., Baek, H.H., Meyers, S.P., Cadwallader, K.R. and Godber, J.S. 1994. Crayfish hepatopancreatic extract improves flavor extractability from a crab processing by-product. J. Food Sci. 59: 91-96.

Konopka, U.C. and Grosch, W. 1991. Potent odorants causing the warmed-over flavour in boiled beef. Z. Lebensm. Unters. Forsch. 193: 123-125.

Konosu, S., Yamaguchi, K., and Hayashi, T. 1978. Studies on flavor components in boiled crabs--I. amino acids and related compounds in the extracts. Bull. Japan. Soc. Sci. Fish. 44: 505-510.

Konosu, S. and Yamaguchi, K. 1982. The flavor components in fish and shellfish. Ch. 17. In *Chemistry & Biochemistry of Marine Food Products*, R.E. Martin, G.J. Flick, C.E. Hebard, and D.R. Ward (Ed.), p. 367-404. AVI Publishing Co., Westport, CT.

Krishnamurthy, T.G., Smouse, T.H., Mookherjee, B.D., Reedy, B.R., and Chang, S.S. 1967. Identification of 2-pentylfuran in fats and oils and its relationship to the reversion flavor of soybean oil. J. Food Sci. 32: 372-374.

Kubota, K., Uchida, C., Kurosawa, K., Komuro, A. and Kobayashi, A. 1989. Identification and formation of characteristic volatile compounds from cooked shrimp. Ch. 35. In *Thermal Generation of Aromas*, ACS symposium series 409, T.H. Parliment, R.J. McGorrin and C.T. Ho (Ed.), p. 376-385. American Chemical Society, Washington, DC.

Lee, E., Meyers, S.P., and Godber, J.S. 1993. Development and sensory evaluation of a minced meat crabcake from blue crab processing by-products. J. Food Sci. 58: 99-103.

Likens, S.T. and Nickerson, G.B. 1964. Detection of certain hop oil constituents in brewing products. Amer. Soc. Brew. Chem. Proc. p. 5-13.

Liu, R.H., Legendre, M.G., Kuan, J.W., St. Angelo, A.J., and Vercellotti, J.R. 1987. Heteroatomic compounds associated with beef flavor. Ch. 8. In *Warmed-Over Flavor of Meat*, A.J. St. Angelo and M.E. Bailey (Ed.), p. 193-236. Academic Press, Orlando, FL.

Matiella, J.E. and Hsieh, T.C.-Y. 1990. Analysis of crabmeat volatile compounds. J. Food Sci. 55: 962-966.

MacLeod, A.J. and Cave, S.J. 1976. Volatile flavour components of eggs. J. Sci. Food Agric. 27: 799-806.

McGill, A.S., Hardy, R. and Burt, J.R. 1974. Hept-*cis*-4-enal and its contribution to the off-flavour in cold stored cod. J. Sci. Food Agric. 25: 1477-1489.

Meijboom, P.W. and Stroink, T.B.A. 1972. 2-Trans,4-*cis*,7-*cis*-decatrienal, the fishy off-flavor occurring in strongly autoxidized oils containing linolenic acid or ω3,6,9,etc-fatty acids. J. Am. Oil Chem. Soc. 49: 555-558.

Meyers, S.P. 1987. Crawfish--Total product utilization. Infofish Marketing Digest. 3: 31-32.

Meyers, S.P. and No, H.K. 1994. Utilization of crawfish pigment and other fisheries processing by-products. In press.

Min, D.B.S., Ina, K., Peterson, R.J., and Chang, S.S. 1977. The alkylbenzenes in roast beef. J. Food Sci. 42: 503-505.

Moody, M.W. 1974. Louisiana seafood delight--The blue crab. LSU Cooperative Extension Service. Louisiana State University, Baton Rouge, LA.

Moore, D.S. and McCabe, G.P. (Ed.). 1989. Ch. 8. *Introduction to the Practice of Statistics*, 1st ed., p. 541-550. W.H. Freeman and Company, New York, NY.

Morton, I.D., Akroyd, P. and May, C.G. 1960. Flavoring substances. U.S. Patent 2,934,437.

Nieto, M., Beltran, A., and Toledo, R.T. 1989. Extraction and characteristics of flavor concentrate from mechanically recovered crab meat. Presented at 14th Annual Conference of Tropical and Subtropical Fisheries Technological Society of the Americas, Gainesville, FL, Oct 1-4.

Nunomura, N., Sasaki, M., Asao, Y. and Yokotsuka, T. 1976. Identification of volatile components in Shoyu (soy sauce)

by gas chromatography-mass spectrometry. Agric. Biol. Chem. 40: 485-490.

O'Bannon, B.K. (Ed.). 1993a. *Current fishery statistics no. 9200--Fisheries of the United States, 1992*. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Fisheries Statistics Division, Washington, D.C.

O'Bannon, B.K. (Ed.). 1993b. *Current fishery statistics no. 9200--Fisheries of the United States, 1992*, Supplemental. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Fisheries Statistics Division, Washington, D.C.

Okumura, S., Kawanobe, T. and Amaike, M. 1993. Flavoring compositions containing pyrroles for Chinese noodles. Jpn. Kokai Tokkyo Koho Japanese Patent 05 15,320.

Pagano, R.R. (Ed.). 1986. *Understanding Statistics in the Behavioral Sciences*, 2nd ed. West Publishing Co., St. Paul, MN.

Peers, K. and Swoboda, P.A.T. 1977. Volatile odorous compounds responsible for metallic, fishy taint formed in butterfat by selective oxidation. J. Sci. Fd Agric. 28: 1010-1018.

Pippen, E.L., Mecchi, E.P., and Nonaka, M. 1969. Origin and nature of aroma in fat of cooked poultry. J. Food Sci. 34: 436-442.

Rayner, E.T., Dupuy, H.P. Legendre, M.G., Grodner, R.M., Cook, J.A., Novak, A.F. and Toloday, D.J. 1981. Instrumental analysis of seafood composition. J. Food Sci. 47: 76-78.

Roessler, E.B., Pangborn, R.M., Sidel, J.L. and Stone, H. 1978. Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests. J. Food Sci. 43: 940-944.

SAS Institue, Inc. 1989. *SAS/STAT© User's Guide*, Vesion 6, 4th ed. SAS Institute, Inc., Cary, NC.

Schieberle, P. and Grosch, W. 1987. Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. Z. Lebensm. Unters. Forsch. 185: 111-113.

Schmitt, W.L. (Ed.). 1965. *Crustaceans*. The University of Michigan Press. Ann Abbor, MI.

Seals, R.G. and Hammond, E.G. 1970. Some carbonyl flavor compounds of oxidized soybean and linseed oils. J. Am. Oil Chem. Soc. 47: 278-280.

Shibamoto, T. 1989. Volatile flavor chemicals formed by the Maillard reaction. Ch. 13. In *Thermal Generation of Aromas*, ACS symposium series 409, T.H. Parliment, R.J. McGorrin, and C.T. Ho (Ed.), p. 134-142. American Chemical Society, Washington, D.C.

Shibamoto, T. and Russell, G.F. 1976. Study of meat volatiles associated with aroma generated in a D-glucose-hydrogen sulfide-ammonia model system. J. Agric. Food Chem. 24(4): 843-846.

Shye, S.-C., Pan, B.S., and Wu, C.-M. 1987. Preliminary identification of flavor components extracted from shrimp (*Parapenaeus fissurus*). J. Chinese Agric. Chem. Soc. 25:169-176.

Siddiquie, P.J.A., Akbar, Z. and Qasim, R. 1987. Biochemical composition and calorific values of the three edible species of portunid crabs from Karachi. Pakistan J. Sci. Ind. Res. 30: 119-121.

Srinivas, S.R. 1988. Volatile constituents in alfalfa and red clover extracts. Dev. Food Sci. 18: 343-353.

Stahl, W.H. (Ed.). 1973. *Compilation of Odor and Taste Threshold Values Data*. ASTM data series DS48. American Society for Testing and Materials, Philadelphia, Pa.

Swoboda, P.A.T. and Peers, K. 1977. Metallic odour caused by vinyl ketones formed in the oxidation of butterfat. The identification of Octa-1-*cis*-5-dien-3-one. J. Sci. Food Agric. 28: 1019-1024.

Tanchotikul, U. and Hsieh, T. C.-Y. 1991. Analysis of volatile flavor components in steamed Rangia clam by dynamic headspace sampling and simultaneous distillation and extraction. J. Food Sci. 56: 327-331.

Tanchotikul, U. and Hsieh, T.C.-Y. 1989. Volatile flavor components in crayfish waste. J. Food Sci. 54: 1515-1520.

Tressl, R. 1990. Processed flavors--scope and limitations. Ch. 2. In *Flavour Science and Technology*, Y. Bessiere and A.F. Thomas (Ed.), p. 87-104. John Wiley & Sons Ltd., New York, NY.

Ullrich, F. and Grosch, W. 1987. Identification of the most intense volatile flavour compounds formed during

autoxidation of linoleic acid. Z. Lebensm. Unters. Forsch. 184: 277-282.

van den Dool, H. and Kratz, P.D. 1963. A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. J. Chromatogr. II: 463-471.

van den Ouweland, G.A.M. and Peer, H.G. 1975. Components contributing to beef flavor. Volatile compounds produced by the reaction of 4-hydroxy-5-methyl-3(2H)-furanone and its thio analog with hydrogen sulfide. J. Agric. Food Chem. 23(3): 501-505.

Vejaphan, W., Hsieh, C.-Y., and Williams, S.S. 1988. Volatile flavor components from boiled crayfish (*Procambarus clarkii*) tail meat. J. Food Sci. 53: 1666-1670.

Viro, M. 1984. N-Heterocyclic aroma compounds in whiskey. Found. Biotech. Ind. Ferment. Res. 3: 227-33.

Warner, G.F. (Ed.). 1977. *The Biology of Crabs*. Van Nostrand Reinhold Company, New York, NY.

Wasserman, A.E. and Talley, F. 1968. Organoleptic identification of roasted beef, veal, lamb and pork as affected by fat. J. Food Sci. 33: 219-223.

Whitfield, F.B., Freeman, D.J., and Bannister, P.A. 1981. Dimethyl trisulphide: an important off-flavour component in the royal red prawn (*Hymenopenaeus sibogae*). Chemistry and Industry. p. 692-693. October 3.

Whitfield, F.B., Last, J.H., Shaw, K.J. and Tindale, C.R. 1988. 2,6-Dibromophenol: The cause of an iodoform-like off-flavour in some Australian Crustacea. J. Sci. Food Agric. 46: 29-42.

Widder, S., Sen, A. and Grosch, W. 1991. Changes in the flavour of butter oil during storage--identification of potent odorants. Z. Lebensm. Unters. Forsch. 193: 32-35.

Yamada, K. 1967. Occurrence and origin of trimethylamine oxide in fishes and marine invertebrates. Bull. Japan. Soc. Sci. Fish. 33: 591-603.

Yang, X.H., Lee, C. and Scranton, M.I. 1993. Determination of nanomolar concentrations of individual dissolved low molecular weight amines and organic acids in seawater. Anal. Chem. 65: 572-576.

## APPENDIX A. EXAMPLE OF A FORM FOR CRAB TASTE EVALUATION

Name:_____

I. Can you tell the difference in taste of these soups?

**********
*                                                *
*Try to breath through your mouth and NOT your nose*
*                                                *
**********

Method:

1. Beware of a spill.
2. All "Crab soup" were prepared from crabmeat.
3. Rinse your mouth with water each time you taste a sample.
4. Don't swallow! Pour adequate amount of soup, 1 cc., into your mouth. Let the soup spread on your tongue, and your throat.
5. Pick the tube that tastes different from each set and circle that tube number below.

---

| | | | |
|---|---|---|---|
| Set 1. | 874 | 015 | 374 |
| Set 2. | 739 | 985 | 185 |
| Set 3. | 689 | 391 | 233 |
| Set 4. | 145 | 453 | 946 |

Describe the differences, if any:

## APPENDIX B. EXAMPLE OF A FORM FOR CRAB AROMA EVALUATION

Name:_____

I. Can you tell the difference in AROMA of these soups?

```
**************************************************
*                                                *
*  Hold a sample in your mouth for 3 seconds     *
*  and exhale slowly from your respiratory tract.*
*  Let your exhaling breath sweep the volatile   *
*  aromas when it passes through your mouth and  *
*  exits through your nose.                      *
*         DON'T USE YOUR TASTE BUDS NOW!!!       *
*                                                *
**************************************************
```

Method:

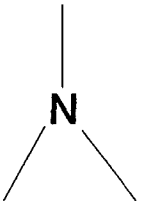
1. Beware of a spill.
2. "Crab soup" was prepared with crabmeat.
3. Rinse your mouth with water each time you taste a sample.
4. Don't swallow! Pour adequate amount of soup, 1 cc., into your mouth. Hold the soup on your tongue.
5. Pick the tube that smells different from each set and circle that tube number below.

---

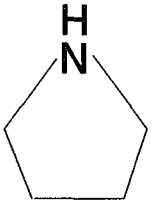
| | | | |
|---|---|---|---|
| Set 1. | 874 | 015 | 374 |
| Set 2. | 739 | 985 | 185 |
| Set 3. | 689 | 391 | 233 |
| Set 4. | 145 | 453 | 946 |

Describe the differences, if any:

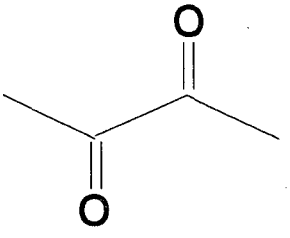
## APPENDIX C. CHEMICAL STRUCTURES OF COMPOUNDS IDENTIFIED IN GAS CHROMATOGRAPHY/OLFACTOMETRY



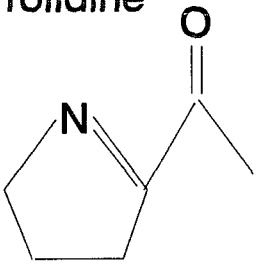

trimethylamine



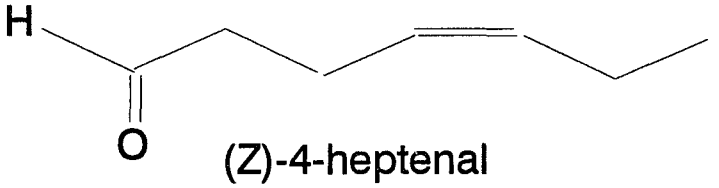

Pyrrolidine



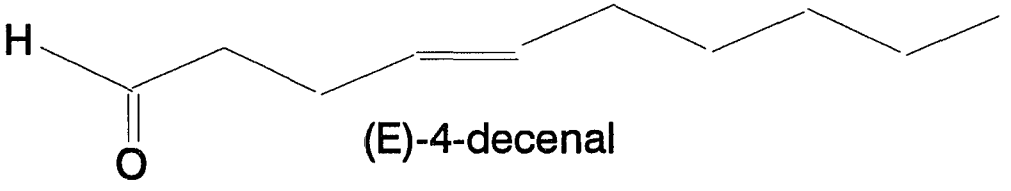

2,3-butanedione



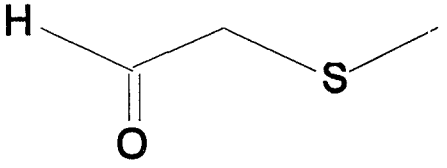

2-acetyl-1-pyrroline




(Z)-4-heptenal




(E)-4-decenal




3-(methylthio)-propanal

## APPENDIX D. EXAMPLE OF A STATISTICAL ANALYSIS SYSTEM PROGRAM FOR DATA ANALYSIS

```
Data heptenal;
input sam ty met trt cl @@;
cards;
1 1 1 1 0.022804 1 1 1 2 0.056354 1 2 2 2 0.026340 1 2 1 2 0.102658
1 1 2 1 0.017573 1 1 2 2 0.027260 1 2 2 1 0.006714 1 2 2 2 0.037421
2 1 1 1 0.031888 2 1 1 2 0.011109 2 2 1 1 0.037780 2 2 1 2 0.163741
2 1 2 1 0.008146 2 1 2 2 0.018087 2 2 2 1 0.005898 2 2 2 2 0.010811
3 1 1 1 0.028344 3 1 1 2 0.110428 3 2 1 1 0.032204 3 2 1 2 0.192371
3 1 2 1 0.010727 3 1 2 2 0.019140 3 2 2 1 0.027898 3 2 2 2 0.034355
4 1 1 1 0.029397 4 1 1 2 0.127558 4 2 1 1 0.036232 4 2 1 2 0.196201
4 1 2 1 0.029862 4 1 2 2 0.015416 4 2 2 1 0.017156 4 2 2 2 0.037177
proc glm;
class sam ty met trt;
model cl = sam ty met trt ty*met ty*trt met*trt ty*met*trt;
lsmeans ty met trt ty*met ty*trt met*trt ty*met*trt/tdiff;
run;
```

# APPENDIX E. SUPPLEMENTARY TABLES

E.1 Proximate analysis (% wet weight) of different species of crab from various parts of the world (from Siddiquie et al., 1987)

| Location | Species | Percent | | | | |
|---|---|---|---|---|---|---|
| | | Moisture | Ash | Protein | Fat | Carbohydrate |
| USA | *Callinectus sapidus* | 1.20 | 1.6 | 16.10 | 1.0 | 1.25 |
| USA | *Cancer magister* | 0.50 | 1.4 | 17.20 | 1.4 | ---- |
| India | *Naptunus* Spp. | 8.40 | 1.45 | 16.50 | 0.5 | 0.30 |
| USA, Japan & India | *Paralithodes camtschatica* | 0.70 | 1.6 | 17.20 | 0.7 | ---- |
| Philippines | *Scylla serrata* | 80.30 | 1.8 | 14.90 | 2.9 | 0.60 |
| Paskistan | *Portunus pelagicus* | 78.06 | 1.78 | 12.35 | 1.02 | 0.48 |
| Paskistan | *P. sanguinotantus* | 78.87 | 2.79 | 13.09 | 1.25 | 0.26 |
| Paskistan | *S. serrata* | 75.53 | 1.44 | 14.78 | 1.29 | 0.59 |

E.2 Proximate composition of cooked blue crab meat (from Hanover et al., 1973)

| Component | % |
|---|---|
| Moisture | 77.08 |
| Protein | 18.42 |
| Fat | 2.48 |
| Ash | 2.02 |

E.3 Volatile compounds identified in blue crab by other researchers (modified from Mateilla and Hsieh, 1990; Flament, 1990; Hsieh et al., 1989; Ando and Osawa, 1988; Rayner et al., 1981)

| Compounds by class | Reference[1] 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Aldehydes | | | | | |
| benzaldehyde | | x | x | | |
| butanal | | x | | | |
| decanal | | | x | | |
| furfural | | | x | | |
| heptanal | x | | x | | |
| hexanal | x | | x | | |
| isovaleraldehyde | | x | | | |
| 3-methylbutanal | x | | x | | |
| octanal | | | x | | |
| pentanal | x | | x | | |
| Ketones | | | | | |
| acetone | | | | | x |
| 2,3-butanedione | x | | | | |
| cyclohexanone | | | x | | |
| 2-heptanone | | | x | | |
| 2-hexanone | x | | x | | |
| 3-hexanone | x | | x | | |
| 2-octanone | | | x | | |
| 2-pentanone | x | | x | | |
| 4-methyl-2-pentanone | x | | | | |

(table con'd)

| | | | | | |
|---|---|---|---|---|---|
| Alcohols | | | | | |
| acetol | | x | | | |
| benzyl alcohol | | x | | x | |
| dodecanol | | | | x | |
| ethanol | | | | | x |
| heptanol | | | | x | |
| methanol | | | | | x |
| phenylethyl alcohol | | x | | x | |
| isoamyl alcohol | | x | | | |
| 1-butanol | x | x | | x | |
| decanol | | | | x | |
| 5-decanol | | | | x | |
| hexadecanol | | | | x | |
| tetradecanol | | | | x | |
| 4-ethyl-2,6-dimethyl-4-heptanol | | | | x | |
| 1-methylcyclohexanol | | | | x | |
| 2-ethyl-1-hexanol | | | | x | |
| 3,5,5-trimethyl-1-hexanol | | | | x | |
| 1-hexanol | | | t | | |
| 2-hexanol | x | | t | x | |
| 3-hexanol | x | | t | x | |
| 1-pentanol | | | t | | |
| 2-propanol | | | x | | |
| isopropanol | | x | | | |
| 2-nonanol | | x | | x | |
| 3-ethyl-3-pentanol | | | | x | |
| 2,5-octadien-1-ol | | | | x | |
| 5-octen-2-ol | | | | x | |
| 7-octen-4-ol | | | | x | |

(table con'd)

| | | | | |
|---|---|---|---|---|
| Aromatics | | | | |
| anisole | x | | | |
| benzene | x | | | |
| 1,4-dichlorobenzene | x | | x | |
| ethylbenzene | x | | x | |
| propylbenzene | x | | x | |
| 1-methyl-2-(2-propenyl)-benzene | x | | | |
| 1,2,4-trimethylbenzene | x | | x | |
| 1,3,5-trimethylbenzene | x | | x | |
| p-cymene | x | | | |
| phenol | | x | x | |
| styrene | x | | | |
| toluene | x | x | | |
| 2-ethyltoluene | x | | | |
| 3-ethyltoluene | x | | x | |
| 4-ethyltoluene | x | | x | |
| m-xylene | x | | x | |
| o-xylene | x | | x | |
| p-xylene | x | | x | |
| Furans | | | | |
| 2-acetylfuran | | x | | |
| 2-ethylfuran | x | | x | |
| 2-pentylfuran | x | | t | |
| Sulfur-containing Compounds | | | | |
| dimethyl disulfide | x | x | x | x |
| methyl mercaptane | | x | | |
| 3,5-dimethyl-1,2,4-trithiolane | | x | | |

(table con'd)

| | | | | | |
|---|---|---|---|---|---|
| Sulfur-containing Compounds | | | | | |
| 2-acetylthiazole | | x | | | |
| methanethiol | | | | | x |
| 2-methylthiophene | x | | x | | |
| dimethyl trisulfide | | x | | | |
| trithiane | | x | | | |
| Terpenes | | | | | |
| limonene | x | | x | | |
| naphthalene | x | | | | |
| Alkanes | | | | | |
| decane | x | | | | |
| nonane | x | | | | |
| undecane | x | | | | |
| Miscellaneous compounds | | | | | |
| acetoin | | x | | | |
| ethyl acetate | | x | | | |
| trimethylamine | | | | | x |
| 2,5-dimethyl-2,4-hexadiene | x | | | | |
| indole | | x | | | |
| dibromomethane | x | | | | |
| trimethyloxazole | | x | | | |
| pyridine | | x | | | |
| pyrrole | x | x | | | |

(table con'd)

| | |
|---|---|
| Pyrazines | |
| pyrazine | x |
| methylpyrazine | x |
| 2,3-dimethylpyrazine | x |
| 2,5-dimethylpyrazine | x |
| 2,6-dimethylpyrazine | x |
| 2-ethyl-3,6-dimethylpyrazine | x |
| 5-ethyl-2,3-dimethylpyrazine | x |
| 2-(1-propenyl)pyrazine | x |
| 2,6-diethyl-3-methylpyrazine | x |
| trimethylpyrazine | x |
| tetramethylpyrazine | x |

[1] 1: Matiella and Hsieh, 1990; 2: Ando and Osawa, 1988; 3: Hsieh et al., 1989.
4: Flament, 1990; 5: Rayner et al., 1981.
x: positively identified; t: tentatively identified.

## VITA

The author was born in Hong Kong on Feb 2, 1963. He graduated from Hong Kong Tang King Po College in May, 1982. In Spring 1983 he entered the University of Hawaii at Manoa. With the aloha spirits in the Department of Food Science and Human Nutrition and within the College of Tropical Agriculture and Human Resources, his intellectual thoughts began to furish. His name was on the Dean's list for his entire undergraduate studies. He also received the Carey D. Miller award from the Hawaii Dietetics Association in 1985, and graduated with distinction in Fall, 1986 in Food Technology.

His intense interest in Food Science prompted him to further his studies in the area of seafood processing by-product utilization. His research was conducted under the guidance of Dr. W.K. Nip at the University of Hawaii at Manoa. His thesis was entitled "Investigation on the Conversion of Shrimp Processing Waste into Flavoring Ingredient." He received his M.Sc.in December 1989.

With the ambition of widening his knowledge at another U.S. university and through the sincere communication with his former advisor, Dr. Thomas C.-Y. Hsieh, he decided to continue his Ph. D. studies at the Louisiana State University. Unfortunately, his advisor resigned a year later. Nevertheless, he continued to make

strides in his interest and managed the analytical flavor laboratory in the department until his present advisor, Dr. Keith R. Cadwallader, took over.

During his entire graduate studies, he supported himself either from a graduate teaching assistantship and/or work as a laboratory assistant. He is a member of the Institute of Food Technologists (IFT), Chinese American Food Society (CAFS) and the America Chemical Society (ACS).

Currently, he is a candidate for the degree of Doctor of Philosophy in Food Science.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

**Candidate:** Mr. Hau Yin Chung

**Major Field:** Food Science

**Title of Dissertation:** Instrumental and Sensory Characterization of the Flavor of Blue Crab (*Callinectes sapidus*) Meat and Processing By-Product

**Approved:**

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Robert M. Grodner

J. Samuel Godber

**Date of Examination:**

March 21, 1994